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EFFECTS OF FEEDING EMULSIFIERS ON

REPRODUCTION OF C57BL/6 MICE

by

Homer Donald Paschall

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Dean of Graduate College

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INTRODUCTION

Partial acid esters of sugar alcohol anhydrides, their polyoxythylene derivatives, and the polyoxyethylene derivatives of fatty acids are widely used as surface active agents and emulsifiers. During the past several years there has been an increase in the introduction of some of these additives in various food products, regularly consumed by human beings, such as bakery goods, ice cream, confections, salad dressings, and cream and dessert toppings in pressurized cans. Because of this increased usage, there has been considerable interest concerning the possible effect these substances might have upon the consumer. Several studies with laboratory animals have been conducted, but the results have not been in agreement. Poling *et al*. (1956) reported that rats fed polyoxyethylene sorbitan monolaurate at the 25% level developed several gross pathological changes; and, when fed at the 10% level, most of the male rats showed decreased spermatogenesis. Schweigert *et al*. (1950) fed two polyoxyethylene monosterate compounds to weanling hamsters. They concluded that these products, when fed at the 5% or 15% levels, caused a significantly reduced rate of weight gain. However, Krantz *et al*. (1952) reported that these products failed to have adverse effects when fed to rats, monkeys, dogs and mice. According to Brush *et al*. (1957), the addition of certain surface-active agents, including *Tween 60*, at concentrations of 2.5%, 10% and 15% to the diets of hamsters, mice or dogs did not alter the growth or food efficiency of these animals.

Some of the early investigations, like those of Sherman *et al*. (1950), Harris *et al*. (1951), and Chow *et al*. (1953), of these emulsifiers have been conducted over a relatively short period of time extending through only part of one generation of the experimental animal's life span. Therefore, in view of the existing disagreement concerning the effects of these materials on the consumer, and the relative lack of information of their possible toxicity, the present investigations were undertaken. The purposes of this study were to determine what influences long-term, continuous feeding of selected emulsifiers might have upon the reproductive performance of C57BL/6J strain mice. Their reproductive performance was measured by criteria such as average litter size, number per litter born dead, number per litter that survived to weaning, and average weight per mouse at weaning. Results were recorded for control mice of different ages, and for experimental mice of different ages and with varying lengths of exposure to the contaminated diets. All weights are given in grams unless otherwise indicated.

The two emulsifiers chosen for investigation were polyoxyethylene sorbitan monolaurate and polyoxyethylene sorbitan monosterate, which, in the body of the dissertation, are referred to by the commercial trade names of Tween 20 and Tween 60, respectively.

REVIEW OF LITERATURE

There have been many studies which involved the reproduction of mice. As Strong (1950) noted, mice are the smallest and fastest breeding mammal readily available for experimental purposes. Jones and Krohn (1961) pointed out that levels of fertility differ among strains of mice, and Roderick[1] cautioned that even mice of the same strain would show variations due to differences in environmental conditions found in one laboratory as compared with those in another. Because of the many possible variations in mice, the present literature review has been restricted to the more general aspects of mouse reproduction with special reference to the C57BL/6 strain.

Many studies have been conducted which involved the use of food emulsifiers. The section of the literature review dealing with food emulsifiers has been restricted to their more general use, and to their effects, with special attention being given to those of Tween 20 and Tween 60.

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[1]Roderick, Thomas H., Bar Harbor, Maine. Variations in levels of fertility. Private communication. 1961.

Mouse Reproduction

According to Snell (1941) the estrous cycle of mice is about 5 days and the gestation period usually 19 or 20 days. Strong (1950) noted that mice may be weaned at the age of 21 days at which time the young may weigh over 10 grams. Strong (1950) also gave some information relative to the estrous period which follows shortly after the offspring have been delivered. If mating takes place at this time, it is possible for the female again to become pregnant so that the processes of lactation and gestation proceed simultaneously. If the second pregnancy begins shortly after delivery, implantation is delayed and the young of the second litter may be delivered as late as 26 days rather than the average 19 or 20 days. If pregnancy does not occur at the post partum estrous, the next estrous cycle does not take place until the young are weaned.

Jones and Krohn (1961) recorded that there are distinct strain differences in ages at which mice become sexually mature. Russell (1954) stated that most females deliver their first litter before the age of 3 months, and that in the C57BL/6 mice, the average age is 88.5 days.

According to Snell (1941) the useful breeding period of most inbred mice terminates when they reach 10 to 12 months of age. The general concept that the reproductive ability of mice decreases as mice become older is supported by Murray (1934) who recorded that after the age period 200 to 229 days, there is a general decline in fertility. Jones and Krohn (1961) acknowledged a decline in litter size which occurs with advancing age. Data reported by Little and Pearsons (1940) also support the concept. Mole (1959) stated that aging reduces reproductive capacity.

Hauschka (1952) mentioned that with increasing age there was a progressive deterioration in maternal care. Jones and Krohn (1961) expressed the idea that decline in litter size as the females get older is due to increased embryonic mortality in later litters and not to the change in the number of oocytes or the decline in the number of Graafian follicles. They suggest that the decline in fertility toward the end of the reproductive life span may be due to defects in hormonal control of the ovaries, or in the uterine environment. Little and Pearsons (1940) noted an increased interval between pregnancies after the female had delivered 5 litters.

Another characteristic generally noted in mouse reproduction is that the first litter is smaller than the second and that either the second or third litter is the largest. This statement is supported in part or entirely by Watt (1934), Little and Pearsons (1940), Bruce (1947), and Russell (1954).

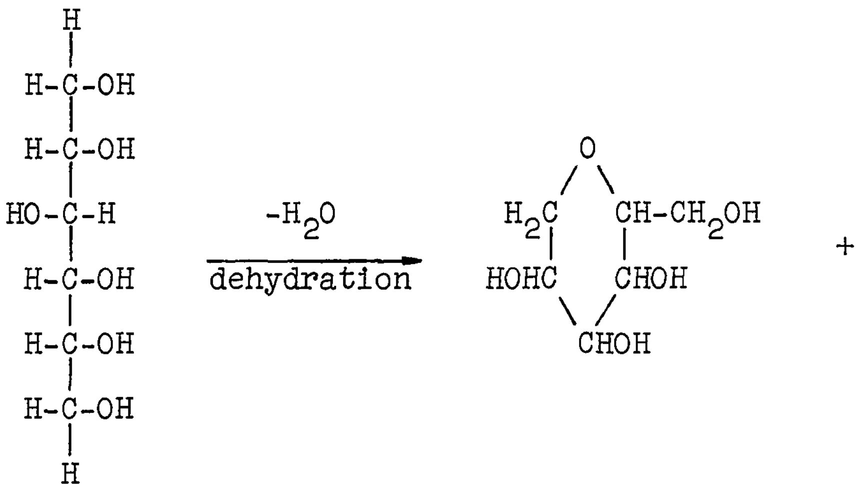
In addition to the decrease in size of the litter with age, there is an increase in the number of offspring born dead. Records of Little and Pearsons (1940) showed a rather marked increase in the number of mice born dead to C57BL mice after the females reached 274 days. Murray (1934) stated that after the third litter, the percentage of offspring dead at birth is very likely to be larger than in the first three litters. He also noted that there is less mortality among the young of litters born while the mother is in the early period of sexual maturity. The data reported by Little and Pearsons (1940) showed a rather high percentage of mice dead at birth in the first litter.

Food Emulsifiers

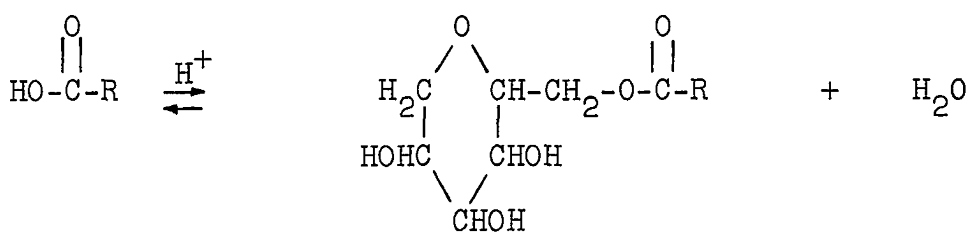
The polyoxyethylene derivatives of fatty acids and the polyoxyethylene derivatives of partial fatty esters of sugar alcohol anhydrides are used as surface active agents and emulsifiers. Schwartz and Perry (1949) noted that one of the best known and widely used series of non-ionic water soluble surface active agents is produced by the Atlas Powder Company and sold under the trade name, *Tween*.

Krantz *et al.* (1948) described how a *Tween* could be produced. The following description is based upon the information found in their report. Sorbitol is first dehydrated to yield anhydrosorbitol (sorbitan). Anhydrosorbitol is then esterified with a fatty acid such as lauric, palmitic, oleic or stearic. The resulting ester is etherified with ethylene oxide. This can be represented by the following chemical sequence:

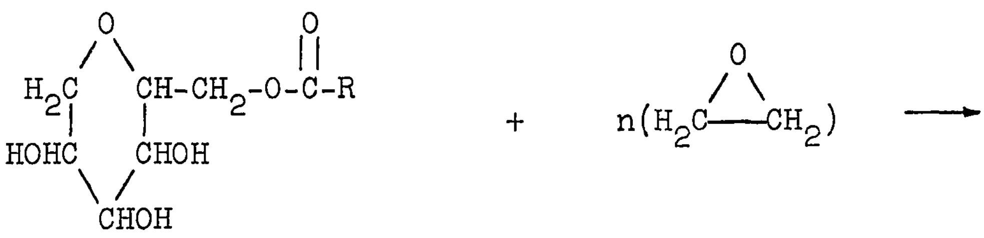
Sorbitol $-$ H_2O $\longrightarrow$ anhydrosorbitol (sorbitan) +



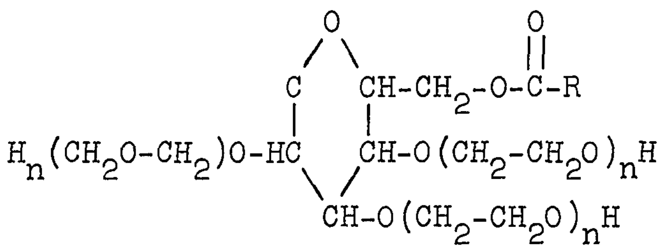
a fatty acid ester of anhydrosorbitol + water



Ester of anhydrosorbitol + Ethylene oxide ⟶

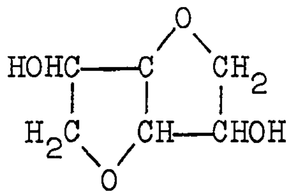


A <u>Tween</u>

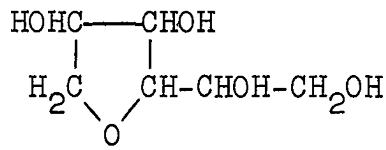


The number of ethylene oxide units and the type of fatty acid combined with the anhydrosorbitol vary from one <u>Tween</u> compound to another. For example, there are approximately 20 ethylene oxide units in <u>Tween</u> <u>20</u> and the fatty acid is lauric acid.

Schwartz and Perry (1949) stated that sorbitol is produced by the hydrogenation of glucose. They also called attention to the fact that there may be more than one form of anhydrosorbitol. In addition to the one shown above, the following two were noted:



and



Each of the anhydrosorbitols contains at least two hydroxyl groups, one of which is available for esterification with the fatty acid and the other for etherification with ethylene oxide. Anhydrosorbitol esters which have not been etherified are sold under the trade name Span.

General use

Pratt and Hays (1952) noted that emulsifiers improve eating quality, appetizing nature and attractiveness of many foods. Emulsifiers help to distribute oil soluble ingredients homogeneously throughout the product. Some of the general functions of emulsifiers are to retard firming and give better texture to bread, make ice cream dryer and stiffer, improve texture of confectionery products, and to disperse flavor in pickles and soft drinks. Tween 60 has been used to assist in the formation and stabilization of pressurized cream products; to control fat bloom on chocolate-covered products; to reduce crystallization time for sugars; and to reduce the tendency for chewy caramels to stick to teeth. Tween 20 has been used to disperse the flavor in hard candies, pickles and soft drinks, and to disperse fat soluble vitamins in aqueous media.

Easton *et al*. (1952) reported Tween 60 in combination with sorbitan monosterate as being helpful in retarding fat bloom in chocolate.

Tween 80 was reported by Synderman *et al*. (1953) to increase slightly fat and vitamin A assimilation in premature infants. Krantz and Carr (1961) stated that Tween 80 was useful in promoting the absorption of fat from the alimentary tract in certain pathological conditions as sprue and celiac diseases.

Deleterious effects of feeding certain food emulsifiers, such as the Tween compounds, to experimental animals has been reported by several workers. Harris *et al*. (1951) reported that hamsters fed sorbitan monlaurate, polyoxyethylene monolaurate, or polyoxyethylene sorbitan monolaurate (Tween 20) showed a poorer weight increase and less efficient utilization of the diet than did animals fed the control diet. In addition to poor weight increase, they reported a higher mortality rate among the animals receiving each of the experimental diets. Histopathological results indicated that polyoxyethylene sorbitan monolaurate, as well as the other test compounds, irritated the gastrointestinal tract and inhibited gonadal maturation. The experimental diets contained 5% or 15% of the emulsifier. A report by Sherman et al. (1950) stated that polyoxyethylene sorbitan monolaurate was toxic to hamsters when fed at 5% and 15% of a nutritionally adequate diet.

Eagle and Poling (1956) reported adverse effects on hansters fed polyoxyethylene sorbitan monolaurate at a 10% level. They also reported that it was common for animals on the experimental diet to have small testes and enlarged caeca.

Poling et al. (1956) raised rats on diets which contained from 5% to 25% Tween 20. In general, the rats fed the experimental diets showed retarded growth, increased mortality and consistent diarrhea.

The results of an experiment by Harris et al. (1951) indicated that certain emulsifiers, including polyoxethylene sorbitan monolaurate, were toxic to rats when fed at the 25% level. Eagle and Poling (1956) reported that 38% of the rats fed diets containing polyoxyethylene sorbitan monolaurate at the 25% level had small testes.

According to Meng and Freeman (1948) the injection of Tween 20 in dogs brought about such toxic manifestations as dilation of blood vessels; decrease in blood pressure; vomiting; and urticaria. They suggested that the effect was due to histamine formation in the body. According to Krantz et al. (1948) man appears to enjoy an immunity to this allergic type of response to similar compounds.

Schweigert et al. (1950) noted a retarded growth rate in weanling hamsters when polyoxyethylene monosterate products were fed at levels of 5% and 15%.

Some workers, such as Ershoff and Hernandez (1959), Allison et al. (1952), and Chow et al. (1953), reported that deleterious effects of feeding some of the surface active agents can be altered by the type of food ingested with the surface active agent. Certain bulky foods such as agar gel, alfalfa meal, and dehydrated rye, orchard, wheat, and fescue grasses reduced the toxic effects.

METHODS

Selection and Care of Animals

Immature stock mice, 4 to 5 weeks of age, of the C57BL/6J strain were obtained directly from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. To insure continuance of the highly inbred strain, a pattern of brother-sister mating was planned in conformity with the routine followed at the Jackson Memorial Laboratory. After the mice reached approximately 8 weeks of age, they were allowed to breed. Offspring of these matings were used as the first generation animals for this study.

Mice were housed in a well-ventilated, air-conditioned, controlled temperature (74 ± 2 F^{o}) animal room. Cages were of clear plastic (14 x 5 x 6 inches) with perforated stainless steel lids. Wood shavings served as bedding. Plastic glasses with No. 11.5 rubber stoppers and stainless steel tubes served as water dispensers. Food was kept in stainless steel food cups. As a rule each breeding female was housed in an individual cage. Five males were housed in one cage except when they were being used for breeding purposes, or when fighting made it necessary to separate them. Bright sunlight was excluded from the laboratory by the use of black shades. The animals were exposed to strong artificial light only during the time in which they were being cared for or checked.

Purina Mouse Breeder Chow[1] in mash form was the control diet. The experimental diets were prepared by mixing the Purina Mouse Breeder Chow with a sufficient quantity of one or the other of the polyoxyethylene preparations to produce feed containing a 10% concentration. To insure uniformity in the test diets, 900 grams of the finely ground chow were thoroughly mixed with 100 grams of the emulsifier. A uniform distribution was obtained by first hand mixing, followed by mechanical mixing for a period of about 10 minutes. In the body of the dissertation, the experimental diets are referred to as Tween 20 (polyoxyethylene sorbitan monolaurate) and Tween 60 (polyoxyethylene sorbitan monosterate) diets. The mice were fed *ad libitum* with the exception of groups 2, 4, 5 and 6 which were deprived of access to food over night

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[1]Percentage composition as furnished by the manufacturer:

Mouse Breeder Chow

Crude protein (not less than)	17%
Crude fat (not less than)	11%
Crude fiber (not more than)	2%
N. F. E. (nitrogen free extract)(not less than)	52%
Ash (not more than)	5.5%

Ingredients: Dried skimmed milk, ground wheat, brewer's yeast, corn oil, animal fat (preserved with butylated hydroxyanisole), Vitamin A feeding oil, D activated plant sterol, 1.4% salt, .13% iron citrate.

during the time, as described later, when males and females with different dietary histories were put together for breeding.

Each week, animals were transferred to clean cages with new wood shavings. Food cups and water dispensers were replaced with clean ones. Used cages, cups, lids and water dispensers were washed in hot detergent water, rinsed in distilled water, dipped in a disinfectant (Hytron) and drained.

When mice from the stock colony were weaned at 21 days, they were earmarked and placed in one of the seven breeding groups listed in Table 1. Groupings were made in such a way as to make possible the continuance of brother-sister mating. The only exception was in group 5 in which some of the sibling males died and were replaced by closely related males. Each group contained approximately 15 breeding combinations.

When mice reached the age of 11 weeks ± 5 days, the females were removed to individual cages and the first breeding was started. In groups 1, 4 and 7 a male was kept in the cage with the female until she showed such definite signs of pregnancy as an enlarged abdomen or gain in weight. The male was then returned to his original cage. In groups 2, 3, 5 and 6, in which the males and females were on different diets, it was necessary to alter the above procedure.

Table 1. Breeding groups of first generation mice

Group	Diet of male	Diet of female
1	10% Tween 20	10% Tween 20
2	10% Tween 20	Control
3	Control	10% Tween 20
4	10% Tween 60	10% Tween 60
5	10% Tween 60	Control
6	Control	10% Tween 60
7	Control	Control

In these groups, food was removed from the cage of the female in the evening (9-10 P.M.) at which time the male, who had been on his particular diet, was placed in the cage with the female. In the morning (8-9 A.M.), the male was returned to his cage and the food was put back into the female's cage. This daily transfer of the male was continued until the female showed signs of pregnancy. The cage of each pregnant animal was checked each morning and evening for new-born mice. When young were found, records were made relative to the number delivered, the number alive and the number dead. Beginning with the second litter, a count was taken of the number of young that were still alive at the end of 4 days. The young were weaned at 21 days. At that time records were made of the number weaned and, in most cases, notations were made relative to the number of males and females in the group weaned.

At weaning of the first litters from the first generation mice, an apparent difference was noted between the size of the offspring from mothers on the experimental diets, and the size of those from mothers fed the control diet. Because of this initial observation, weights were taken of all subsequent litters at the time of weaning.

In order to investigate the possible cumulative effects of feeding Tween preparations, offspring were taken from groups 1, 4 and 7, earmarked, and placed in cages containing the proper feed which was the same as that of their parents. When these mice reached 10 weeks of age ± 5 days, females were placed in individual cages and the first breeding started. The same procedure was followed in the second generation as was described for groups 1, 4 and 7 of the first generation. When the offspring of the second generation reached weaning age, approximately 13 breeding combinations (usually pairs) were set aside for a third generation breeding. The same procedure was followed for the third generation as was noted for the second generation.

The following procedure was carried out in all groups.

The young were weaned at 21 days. One week after the young were weaned, the same male, if possible, that fathered the previous litter was returned to the female's cage for the next breeding. With the exception noted for groups 2, 4, 5 and 6, the male stayed with the female until she showed signs of pregnancy. In those cases in which all the young were dead at birth or destroyed or died during the first four days, a 2 week period was allowed before the next breeding.

Mice used for the second and third generations were taken from the first litters of the previous generation. For example, mice used as the second generation mice on 10% Tween 20 diet were taken from the litters of those in group 1 (both parents on 10% Tween 20) of the first generation. After the desired number of mice was obtained for the second generation, all excess offspring of the first generation not needed for experimental purposes were killed at the time of weaning.

During the course of this study 4 litters were delivered by the first and second generation females and 3 litters by the third generation mice. More than 4 litters were delivered by some of the first generation mice; however, because only a few of each group were bred more than 4 times, and because some of the mice had reached an age characterized by lowered reproductive potential (Russell, 1954), these data were not analyzed with those from the first 4 breedings but are discussed separately in the Fifth Breeding section under Results and Discussion.

At the end of the experimental period, a sample of the first generation animals was weighed and killed. Gonad weights were recorded. The rate of sperm movement was also measured in samples of seminal fluid from males.

Procedure for Determining Gonad Weights

Ovaries: Females were killed by use of chloroform. Ovaries, removed through an incision into the abdominal cavity, were observed with a binocular microscope in order to remove adhering tissues. Each ovary was then transferred to a chain balance for weighing. Weights were taken to 0.1 milligram.

Testes: To avoid any effect chloroform might have on sperm motility, males were killed by cervical dislocation. Each testis was carefully removed, cleaned, and transferred to a chain balance for weighing to 0.1 milligram.

Procedure for Checking Rate of Sperm Movement

The male was killed as noted above and an incision was made through the abdominal wall in order to remove a testis with its vas deferens. The vas deferens was stripped by use of small forceps. Contents of the vas deferens were emptied into the concavity of a depression slide containing 0.2 ml. of mammalian sperm diluting fluid. This diluent was like that of Weisman's (1941) with the exception that $Na_2HPO_4 \cdot 7H_2O$ was used in place of $Na_2HPO_4 \cdot 12H_2O$. The diluting fluid was warmed to a temperature of 37°C. until it was transferred to the depression slide which was kept on a warming plate set at 37°C. After the contents of the vas deferens had been mixed with the diluent, a drop of the mixture was transferred to a regular microscope slide which had also been kept on the warming plate. A cover glass, supported by a small piece of glass, was placed on the mixture. The slide was then transferred to a microscope warm stage kept at 37°C. and observed under 100 magnification. A calibrated optical micrometer was used to observe the path of movement. Ten sperm were randomly selected and checked for speed. In order to measure the rate of movement, it was necessary to select a sperm that was traveling in a relatively straight line across the micrometer scale. By this means, it was possible to measure the time taken by a spermatozoan to move one large unit along the micrometer scale. The average rate of movement of the sperm of each mouse and the average rate of sperm from all males on one diet were determined. The results are discussed in the Rate of Sperm Movement section under Results and Discussion.

Statistical Methods

Since there were unequal numbers of litters in some of the major groups of mice, an approximate method of statistical analysis was employed. The method used was that for analysis of unweighted means as described by Snedecor (1956). By this procedure, the number of offspring born, the number of offspring alive at 4 days and the number of offspring weaned were evaluated. The overall experimental error used in these analyses was estimated from variation in performance found in each of the cells from which the means were calculated.

The number of offspring born dead was evaluated by the analysis of variance procedure. In this analysis the measure of response was the average number of offspring born dead per litter. Weaning weights of the offspring were evaluated by the method of analysis of covariance in which the number weaned was used as the covariant.

In each of the analyses noted above, the factors partitioned in the analysis of variance were: litter number, treatment, generation and the corresponding interactions between them. When significant differences were demonstrated in the analyses of variance, the test described by Duncan (1955) was sometimes employed to compare the means.

The term litter number was used to identify the sequence of litters delivered by the female. Litter 1 was the first litter delivered by the females. Litter 2 was the second litter delivered, etc.

The term treatment was used to denote the type of diet fed the animals. The first generation mice were divided into 7 treatment groups as outlined in Table 1. There was no suitable way by which diets could be selected for offspring of those groups of mice in which males and females were on different diets. From groups 1, 4 and 7, in which males and females were on the same diet, offspring used for the next generation matings were selected and continued on the same diet.

Because of limits on the duration of the study only 3 litters of mice from the third generation mice could be included. Any comparisons involving 4 litters of mice could only be made between first and second generation mice. The practice of recording the number of offspring alive at 4 days as well as the weaning weights of offspring was not begun until the second litter of the first generation mice. Any comparisons relative to the number of offspring at 4 days or to weaning weights that involved the first litter could be made only between second and third generations. As noted above, the third generation mice delivered only 3 litters so any comparison between the weaning weights of mice in all 3 generations was restricted to the second or third breeding.

In order to make as complete analysis as possible and at the same time have balanced analyses, it was often necessary to perform more than one analysis involving the same data.

RESULTS AND DISCUSSION

Number of Offspring Born

The number of offspring delivered by the 7 groups of first generation mice is recorded in Table 2. In Table 3 is recorded the number of offspring delivered by the first, second, and third generation mice in which both parents were on the same diet. The summary of the analysis of these data is recorded in Tables 4, 5 and 6.

Analysis of data relative to the number of offspring produced by the 3 treatment groups in 3 breedings for each of the 3 generations (Table 6) showed that litter number and treatment had significant effects ($P < .01$ and $P < .05$) and that generation effect was significant at the .10 level. All the analyses were in agreement relative to the significant effect of litter number. In all groups the first litter was typically the smallest with either the second or third being the largest. These findings were consistent with those of Bruce (1947), Russell (1954), and Roderick.[1] The average litter sizes for the control mice of this study were greater than those reported by Russell (1954), but approximately the

[1]Roderick, Thomas H., Bar Harbor, Maine. Data from Roscoe B. Jackson Memorial Laboratory. Private communication. 1961.

Table 2. Number of offspring born to first generation mice

Group 1 20♀ x 20♂ litters				Group 2 C♀ x 20♂ litters				Group 3 20♀ x C♂ litters				Group 4 60♀ x 60♂ litters			
1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
9	-	-	-	-	6	8	6	6	6	9	7	5	10	2	6
6	6	7	10	-	7	7	10	5	10	-	-	6	6	11	7
7	7	11	5	7	8	6	7	6	10	7	-	-	5	6	8
5	-	5	-	11	11	5	-	8	9	9	8	5	5	8	10
4	7	6	9	3	9	4	5	5	6	12	7	5	3	2	7
3	4	4	7	8	10	8	8	6	7	6	8	1	6	7	7
4	3	-	5	10	7	9	8	5	-	9	5	6	8	11	9
6	7	10	9	5	11	12	-	6	11	6	7	7	-	10	-
6	7	8	-	4	9	7	7	7	8	7	9	6	8	8	8
3	6	5	6	6	1	6	8	8	6	10	3	9	10	5	8
5	6	8	6	8	8	3	8	2	5	4	5	8	9	9	-
3	7	6	8	5	8	-	-	6	6	9	7	8	6	10	7
6	7	-	-	3	7	8	3	4	-	8	10	6	9	9	3
5	6	8	7	8	8	9	-	8	12	8	12	9	9	7	6
				5	10	9	-	8	8	5	4	5	7	9	5
				3	2	5	7	-	7	2	10	6	-	10	10
Total															
72	73	78	72	86	122	106	77	90	111	111	102	92	101	124	101
Average															
5.14	6.08	7.09	7.20	6.14	7.63	7.07	7.00	6.00	7.93	7.40	7.29	6.13	7.21	7.75	7.21
Average of averages															
6.379				6.959				7.154				7.078			

Table 2. (Continued)

Group 5 C♀ x 60♂ litters				Group 6 60♀ x C♂ litters				Group 7 C♀ x C♂ litters			
1	2	3	4	1	2	3	4	1	2	3	4
6	7	10	-	-	2	7	6	8	9	8	8
5	8	8	6	6	8	7	7	2	10	9	7
3	8	10	7	2	6	8	7	7	-	-	-
7	6	6	8	7	8	10	7	4	8	6	5
5	4	8	7	5	5	4	-	7	8	9	8
8	11	7	7	5	-	-	-	6	7	9	8
8	6	10	8	5	4	-	-	6	-	-	-
2	8	4	-	7	9	7	9	6	10	10	-
11	5	6	7	2	2	4	7	6	7	8	4
4	5	-	-	6	9	6	8	10	9	8	8
8	9	10	-	10	4	4	8	5	9	7	8
8	9	10	12	7	4	8	-	9	8	9	8
-	11	-	10	4	7	3	-	6	8	8	8
8	7	10	-	3	3	-	-				
7	9	5	9	7	7	6	-				
7	9	11	9	9	5	-	9				
5	9	5	3								
Total											
102	131	120	93	85	83	74	68	82	93	91	72
Average											
6.37	7.71	8.00	7.75	5.67	5.53	6.17	7.56	6.31	8.46	8.27	7.20
Average of averages											
7.458				6.231				7.559			

Table 3. Number of offspring born to first, second, and third generation mice fed Tween 20, Tween 60, and control diets

Generation 1 litters				Generation 2 litters				Generation 3 litters			
1	2	3	4	1	2	3	4	1	2	3	4
					Tween 20						
9	-	-	-	8	9	8	9	8	8	7	
6	6	7	10	6	8	8	7	7	5	8	
7	7	11	5	6	6	10	10	6	9	6	
5	-	5	-	4	5	5	-	7	8	7	
4	7	6	9	7	8	11	7	5	6	6	
3	4	4	7	1	2	10	5	6	9	7	
4	3	-	5	5	10	6	9	4	6	9	
6	7	10	9	7	7	1	5	4	8	7	
6	7	8	-	5	5	10	6	6	-	7	
3	6	5	6	7	9	7	10	4	8	6	
5	6	8	6	4	8	8	3	6	8	9	
3	7	6	8	9	8	11	-	6	7	11	
6	7	-	-	4	8	9	7				
5	6	8	7	5	7	6	7				
				2	7	3	6				
				5	-	-	-				
				3	3	4	7				
				4	6	8	8				
Total											
72	73	78	72	92	116	125	106	69	82	90	
Average											
5.14	6.08	7.09	7.20	5.11	6.82	7.35	7.07	5.75	7.46	7.50	
Average of averages											
6.379				6.588				6.902			

Table 3. (Continued)

Generation 1 litters				Generation 2 litters				Generation 3 litters			
1	2	3	4	1	2	3	4	1	2	3	4
					Tween 60						
5	10	2	6	7	8	6	-	5	8	9	
6	6	11	7	7	6	8	10	9	6	7	
-	5	6	8	2	9	-	5	5	10	10	
5	5	8	10	3	8	10	8	6	9	5	
5	3	2	7	3	4	6	6	7	4	10	
1	6	7	7	7	9	9	9	10	11	12	
6	8	11	9	-	7	7	8	5	8	10	
7	-	10	-	3	7	8	8	5	6	7	
6	8	8	8	2	10	6	8	3	8	8	
9	10	5	8	5	7	-	-	5	7	6	
8	9	9	-	8	8	8	-				
8	6	10	7	3	6	8	-				
6	9	9	3								
9	9	7	6								
5	7	9	5								
6	-	10	10								
Total											
92	101	124	101	50	89	76	62	60	76	84	
Average											
6.13	7.21	7.75	7.21	4.55	7.42	7.60	7.75	6.00	7.60	8.40	
Average of averages											
7.078				6.828				7.333			

Table 3. (Continued)

Generation 1 litters				Generation 2 litters				Generation 3 litters			
1	2	3	4	1	2	3	4	1	2	3	4
				Controls							
8	9	8	8	6	8	9	4	7	7	9	
2	10	9	7	7	7	9	4	6	9	7	
7	-	-	-	10	8	10	9	7	8	6	
4	8	6	5	6	8	5	8	4	8	2	
7	8	9	8	6	5	8	1	9	9	7	
6	7	9	8	5	5	5	3	7	-	-	
6	-	-	-	4	6	7	7	7	6	10	
6	10	10	-	6	8	7	8	4	8	10	
6	7	8	4	4	5	9	-	7	8	2	
10	9	8	8	5	9	4	5	7	10	7	
5	9	7	8	5	10	8	-	9	6	-	
9	8	9	8	6	11	8	4				
6	8	8	8	1	7	2	-				
Total											
82	93	91	72	71	97	91	53	74	79	60	
Average											
6.31	8.46	8.27	7.20	5.46	7.46	7.00	5.30	6.73	7.90	6.67	
Average of averages											
7.559				6.306				7.098			

Table 4. Analysis of variance of number of offspring born to first generation mice in 4 litters

Sources	Degrees of freedom	Sum of squares	Mean square	F value
Treatments (T)	6	6.102775	1.017129	2.94*
Litter number (L)	3	9.565481	3.188494	9.21**
T x L	18	4.930389	.273910	.79
Error	348		.34620	
Total	27	20.598645		

**Significant at .01.

*Significant at .05.

Table 5. Analysis of variance of the number of offspring born to first and second generation mice in 4 litters

Sources	Degrees of freedom	Sum of squares	Mean square	F value
Litter number (L)	3	15.279503	5.093168	16.57**
Treatment (T)	2	1.24375	.562188	1.83
L x T	6	3.634531	.605755	1.97
Generation (G)	1	1.115428	1.115428	3.63*
L x G	3	.497040	.1665680	0.54
T x G	2	2.236911	1.118456	3.64*
L x T x G	6	1.371100	.228517	0.74
Error	308		.307277	
Total	23	25.258888		

**Significant at .01.

*Significant at .05.

Table 6. Analysis of variance of the number of offspring born to first, second, and third generation mice in 3 litters

Sources	Degrees of freedom	Sum of squares	Mean square	F value
Litter number (L)	2	18.674563	9.33728	28.13**
Treatment (T)	2	2.104057	1.052028	3.17*
L x T	4	1.725391	.431348	1.30
Generation (G)	2	1.601141	.800570	2.41/
G x L	4	.977335	.244334	.74
G x T	4	1.995043	.498761	1.50
L x T x G	8	1.465798	.183225	0.55
(Error)	(309)		.33194	
Total	26	28.543328		

**Significant at .01.

*Significant at .05.

/Significant at .10.

same as those reported by Roderick[1]. From the analyses found in Tables 5 and 6, it was concluded that litter number had a similar effect in each generation.

In an effort to incorporate the fourth breeding for more than one generation, the data for the first and second generations involving all 4 breedings were subjected to the

[1] Ibid.

analysis of variance. From this analysis no significant effect due to treatment response was found (Table 5). This lack of significance was probably the result of the unusually large number of small litters produced in the fourth breeding of the second generation control mice. The small litters in this group reduced the overall average of the control mice and tended to neutralize the treatment effect which showed up in the other analyses. The small average litter size of this group of mice was probably responsible for the significant ($P < .05$) generation effect noted in Table 5. This small average litter could also account for the fact that there was a significant ($P < .05$) treatment by generation interaction when the first and second generation mice were compared but not a significant ($P > .1$) interaction when the first, second and third generation were compared without incorporating the fourth litter. In the opinion of Roderick,[1] a significant generation effect would not be expected in this strain of mice. Since a generation effect would not be expected and since treatment had approximately the same effect in each generation of the experimental animals, it is the opinion of the writer that the significant value obtained for the generation effect as well as that for the

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[1]Roderick, Thomas H., Bar Harbor, Maine. Personal letter. Private communication. 1962.

generation by treatment interaction was due to chance occurrence.

From the analysis involving first generation mice (Table 4), results indicated that treatment had a significant effect upon the number of offspring born. The Duncan Multiple Range Test was employed (Table 7) and the results revealed that mice of group 1 produced significantly smaller litters than did either mice of group 5 or 7. Both males and females of group 7 were on the control diet while in group 5 the females were on the control diet and the males were fed the Tween 60 diet. These results suggest that Tween 20 had some depressing action relative to the number of offspring produced.

The litter means of the first, second, and third generation mice were compared by the Duncan Multiple Range Test (Table 8) with the results showing that animals on Tween 20 diet produced significantly ($P < .05$) smaller litters than did the control mice.

The average litter sizes of mice fed Tween 60 were usually larger than those fed Tween 20. One exception to this was noted in group 6 of the first generation mice. The results of the Duncan Multiple Range Test showed the average litter size of group 6 mice to be significantly ($P < .05$) smaller than that of group 3. The only difference between this group and any other group in which females were fed the

Table 7. Duncan Multiple Range Test on average litter size of first generation mice[a]

(a) Shortest significant ranges

P:	(2)	(3)	(4)	(5)	(6)	(7)
R_p	.82	.86	.89	.91	.93	.94

(b) Results

Mouse groups	6	1	2	4	3	5	7
Average litter size	6.23	6.38	6.96	7.08	7.16	7.46	7.56

[a]Those means not underscored by a common line are significantly (P < .05) different.

Table 8. Duncan Multiple Range Test on average litter size of experimental and control mice for three generations[a]

(a) Shortest significant ranges

P:	2	3
R_p	.532	.562

(b) Results

Treatment groups	Tween 20	Tween 60	Control
Average litter size	6.479	6.962	7.139

[a]Those means not underscored by a common line are significantly (P < .05) different.

Tween 60 diet was that the males were fed the control diet. On the basis of information now available, there is no valid explanation for this performance.

The analyses in Tables 5 and 6 indicated that treatment had approximately the same effect in each breeding. However, it is interesting to note that when the groups of females on the control diet (groups 2, 5, and 7 of the first generation mice and the control mice of the second and third generation) are considered, with the exception of group 5, all produced their largest average litter with the second breeding. The largest average litter of all female groups on Tween 60 diet was either the third or fourth litter. The third or fourth average litter of females fed Tween 20 was also largest with the exception of those in group 3 of the first generation. To study this further, a check was made of all females that produced 4 countable litters to determine their largest litter. Fourteen females on Tween 20, 11 on Tween 60, and 26 on the control diet delivered their largest litter from either the first or second pregnancy whereas 25 females fed Tween 20, 15 fed Tween 60, and 18 on the control diet delivered their largest litter from either the third or fourth pregnancy. Considering all the experimental females that produced four litters, 38.46% produced their largest litter on the first or second pregnancy while 59.09% of the control females delivered their largest litter on either the first or second pregnancy. These findings suggest that females fed the experimental diets tend to produce their largest litter at a later age than do the females fed the control diet.

Number of Offspring Born Dead

The number of offspring born dead to all first generation mice is recorded in Table 9, and the number born dead to groups of mice in which both parents were on the same diet over three generations is found in Table 10. These data were analyzed by the analysis of variance procedure outlined by Snedecor (1956). The results of the analyses are recorded in Tables 11 and 12. In these analyses the measure of response was the average number of offspring dead per litter. There was no valid error of estimate from the individual litters; therefore, the higher order interaction terms were utilized as the error estimate. This was used on the assumption that a three-way interaction would be negligible. In the analyses of data for the first generation mice, the two-way interaction between litters and treatment was the highest order available, so analysis was made on the assumption that there was no interaction. In the analyses in which a three-way interaction was used as the error estimate, there was a trend toward interaction between litter

Table 9. Number of offspring born dead to first generation mice

Group 1 20♀ x 20♂ litters				Group 2 C♀ x 20♂ litters				Group 3 20♀ x C♂ litters				Group 4 60♀ x 60♂ litters			
1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	-	-	-	-	2	0	1	1	0	2	0	2	1	2	5
0	0	0	0	-	0	0	5	1	0	-	-	0	1	0	1
1	0	1	1	1	0	0	0	0	0	0	-	-	0	5	0
0	-	0	-	0	0	3	-	0	0	0	1	0	1	2	0
0	1	0	2	1	0	1	0	0	0	0	0	1	0	1	0
0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	5
0	1	-	0	4	0	0	0	0	-	0	1	0	2	0	0
0	0	0	1	0	2	0	-	2	1	1	1	0	-	0	-
0	0	0	-	1	0	0	1	0	1	0	0	4	1	0	0
0	0	0	0	0	0	0	4	2	0	0	3	1	0	0	4
0	0	0	0	0	0	2	2	0	0	0	1	0	1	0	-
0	0	1	0	3	0	-	-	0	1	0	0	1	0	1	0
1	0	-	-	1	1	0	2	0	-	1	0	1	0	0	0
0	0	0	0	1	0	0	-	0	0	0	0	2	0	1	0
				2	3	0	-	0	0	0	0	0	0	0	0
				0	2	0	0	-	1	2	1	0	-	0	2
Total															
2	2	2	4	15	10	6	16	6	4	6	9	12	7	12	17
Average															
.143	.167	.182	.891	1.071	.625	.400	1.583	.400	.286	.400	.643	.800	.500	.750	1.214
Average of averages															
.2228				.9199				.4322				.8161			

Table 9. (Continued)

Group 5 C♀ x 60♂ litters				Group 6 60♀ x C♂ litters				Group 7 C♀ x C♂ litters			
1	2	3	4	1	2	3	4	1	2	3	4
1	1	0	-	-	2	0	0	0	0	0	5
0	0	0	0	0	0	0	0	1	2	0	4
3	2	4	0	1	1	0	1	0	-	-	-
0	1	1	0	1	0	0	7	3	0	0	2
0	2	0	0	0	1	2	-	1	1	0	0
0	0	2	0	0	-	-	-	0	0	0	0
0	3	1	2	0	0	-	-	0	-	-	-
0	0	4	-	1	1	0	0	1	1	0	-
0	0	0	0	1	2	4	2	1	0	1	0
4	0	-	-	0	0	0	1	0	0	0	0
0	0	1	-	0	0	1	0	0	1	0	1
1	0	0	0	3	0	0	-	0	0	0	0
-	3	-	0	0	0	3	-	0	0	0	0
0	0	0	0	3	0	-	-				
6	0	0	0	0	0	0	-				
2	2	2	0	0	0	-	0				
0	0	1	0								
Total											
17	14	16	2	10	7	10	11	7	5	1	12
Average											
1.063	.824	1.067	.1538	.667	.467	.833	1.222	.539	.455	.091	1.182
Average of averages											
.7766				.7972				.5664			

Table 10. Number of offspring born dead to first, second, and third generation mice fed Tween 20, Tween 60, and control diets

Generation 1 litters				Generation 2 litters				Generation 3 litters		
1	2	3	4	1	2	3	4	1	2	3
					Tween 20					
0	-	-	-	0	1	0	0	0	3	0
0	0	0	0	0	0	0	0	1	0	0
1	0	1	1	0	0	0	0	0	0	0
0	-	0	-	0	0	0	-	0	0	0
0	1	0	2	0	0	0	0	0	1	0
0	0	0	0	0	0	0	5	0	0	0
0	1	-	0	0	1	0	0	0	0	0
0	0	0	1	1	0	0	0	0	0	0
0	0	0	-	3	0	1	2	0	-	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0	0
0	0	1	0	0	0	0	-	0	1	0
1	0	-	-	0	0	0	0			
0	0	0	0	0	0	0	0			
				0	0	-	0			
				0	-	-	-			
				0	0	0	0			
Total										
2	2	2	4	4	2	1	7	2	5	0
Average										
.143	.167	.182		.222	.118	.059		.167	.455	0
Average of averages for first three litters										
	.1638				.1329				.2071	

Table 10. (Continued)

Generation 1 litters				Generation 2 litters				Generation 3 litters		
1	2	3	4	1	2	3	4	1	2	3
					Tween 60					
2	1	2	5	2	1	5	-	2	0	0
0	1	0	1	1	2	0	0	0	0	0
-	0	5	0	0	1	-	0	0	0	0
0	1	2	0	1	0	0	0	5	1	5
1	0	1	0	0	0	0	0	2	-	0
0	0	0	5	1	0	0	1	0	0	0
0	2	0	0	-	0	0	0	0	0	0
0	-	0	-	0	0	0	0	1	0	0
4	1	0	0	2	0	0	0	3	0	0
1	0	0	4	0	0	-	-	0	0	6
0	1	0	-	0	1	0	-			
1	0	1	0	0	0	4	-			
1	0	0	0							
2	0	1	0							
0	0	0	0							
0	-	0	2							
Total										
12	7	12	17	7	5	9	1	13	1	11
Average										
.800	.500	.750		.636	.417	.900		1.300	.100	1.100
Average of averages for first three litters										
	.6833				.6510				.833	

Table 10. (Continued)

Generation 1 litters				Generation 2 litters				Generation 3 litters		
1	2	3	4	1	2	3	4	1	2	3
				Control						
0	0	0	5	0	0	0	2	0	0	0
1	2	0	4	1	0	0	1	0	0	0
0	-	-	-	0	0	0	0	2	1	0
3	0	0	2	1	0	0	0	0	1	2
1	1	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	-	-
0	-	-	-	0	0	0	0	0	0	0
1	1	0	-	0	0	0	0	0	0	0
1	0	1	0	0	0	0	0	0	0	2
0	0	0	0	1	0	0	0	0	0	0
0	1	0	1	0	0	0	-	0	0	-
0	0	0	0	0	1	0	0			
0	0	0	0	1	0	1	-			
Total										
7	5	1	12	4	1	1	4	2	2	4
Average										
.539	.455	.091		.308	.077	.077		.182	.200	.222
Average of averages for first three litters										
.3613				.1538				.2013		

Table 11. Analysis of variance of the number of offspring born dead to first generation mice in four litters

Source	Degrees of freedom	Sums of squares	Mean square	F value
Litter No. (L)	3	0.803481	0.267827	2.58
Treatment (T)	6	1.500085	0.250014	2.40
T x L	18	1.872784	0.104044	
Total	27	4.176350		

Table 12. Analysis of variance of the number of offspring born dead to the first, second, and third generation mice in three litters

Source	Degrees of freedom	Sums of squares	Mean square	F value
Litter No. (L)	2	0.18185351	0.0909	1.64
Treatment (T)	2	1.6400813	0.8200	14.80**
Generation (G)	2	0.0555959	0.0278	0.50
L x T	4	0.5902892	0.1476	2.66
L x G	4	0.0495989	0.0124	0.22
G x T	4	0.0804040	0.0201	0.36
G x B x T	8	0.4434379	0.0554	

**Significant at .01.

number and treatment. This indicates that the analyses relative to the first generation mice (Table 11) may have F values that are artifically low.

From the analysis involving three generations (Table 12), treatment was shown to have a significant effect ($P < .01$) on the number of mice born dead. The averages of the number of mice born dead per litter in each treatment group for three generations were compared by the Duncan Multiple Range Test. The results of the test (Table 13) showed that the Tween 60 mice had significantly more mice born dead than did the mice on either the Tween 20 or control diet. The treatment effect was not significant ($P > .05$) when only the first generation mice were compared.

Litter number effect was not as significant relative to the number born dead as it was in relation to the number of offspring born, the number alive at 4 days or to the number of offspring weaned. Even though the litter number effect was only significant near the .10 level, a comparison of the averages revealed a trend toward more offspring being born dead in the first and fourth litters as compared with the second and third litters. This is consistent with the data of Little and Pearsons (1940).

Table 13. Duncan Multiple Range Test for number of offspring born dead to experimental and control mice over three generations[a]

(a) Shortest significant ranges

P:	2	3
R_p:	.25599	.26682

(b) Results

Treatment groups	20 x 20	control	60 x 60
Average number born dead per litter	.1626	.2222	.6598

[a]Those means not underscored by a common line are significantly ($P < .05$) different.

Number of Offspring Alive at Four Days

The number of offspring of all first generation mice, alive at the end of 4 days, is found in Table 14. The number from the first, second and third generation parents fed like diets is recorded in Table 15. The analyses concerning the number of offspring alive at 4 days of age were not as extensive as those relative to the number of offspring born since the collection of this information was not begun until the

Table 14. Number of offspring from first generation mice alive at four days

Group 1 20♀ x 20♂ litters				Group 2 C♀ x 20♂ litters				Group 3 20♀ x C♂ litters				Group 4 60♀ x 60♂ litters			
1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
-	6	7	9	-	3	8	3	-	6	0	4	-	3	0	0
-	7	10	2	-	6	7	0	-	10	-	-	-	5	11	2
-	-	5	-	-	8	6	7	-	9	7	-	-	5	1	8
-	5	5	7	-	11	0	-	-	8	8	7	-	4	6	10
-	4	4	7	-	9	3	0	-	5	7	6	-	3	1	3
-	2	-	4	-	10	8	4	-	6	6	7	-	6	7	1
-	7	10	3	-	7	9	8	-	-	9	4	-	6	11	9
-	7	8	-	-	9	12	-	-	10	4	0	-	-	10	-
-	6	3	6	-	9	7	5	-	7	7	9	-	7	8	8
-	6	8	6	-	1	6	0	-	5	9	0	-	9	4	0
-	7	5	8	-	8	0	4	-	5	4	3	-	8	7	-
-	7	-	-	-	8	-	-	-	5	9	7	-	6	9	6
-	6	8	6	-	6	8	0	-	-	7	10	-	7	9	2
				-	8	9	-	-	11	8	0	-	9	4	6
				-	7	8	-	-	8	5	0	-	7	9	5
				-	-	5	6	-	6	0	9	-	-	8	8
Total															
	70	73	58		110	96	37		101	90	64		85	105	68
Average															
	5.83	6.64	5.80		6.88	6.40	3.36		7.21	6.00	4.71		6.07	6.56	4.86
Average of averages															
		6.090				5.546				5.976				5.830	

Table 14. (Continued)

Group 5 C♀ x 60♂ litters				Group 6 60♀ x C♂ litters				Group 7 C♀ x C♂ litters			
1	2	3	4	1	2	3	4	1	2	3	4
-	6	10	-	-	0	7	0	-	9	7	0
-	8	8	6	-	8	3	6	-	8	9	0
-	6	6	7	-	4	7	4	-	-	-	-
-	5	5	8	-	8	9	0	-	7	6	0
-	0	8	7	-	4	2	-	-	7	9	8
-	11	4	5	-	-	-	-	-	6	8	7
-	3	9	6	-	4	-	-	-	-	-	-
-	8	0	-	-	6	6	9	-	8	9	-
-	5	6	6	-	0	0	0	-	6	7	4
-	5	-	-	-	9	6	7	-	8	7	8
-	9	9	-	-	4	3	6	-	8	5	6
-	9	10	12	-	4	8	-	-	8	8	6
-	8	-	10	-	7	0	-	-	8	7	7
-	7	10	-	-	2	-	-				
-	9	5	0	-	7	0	-				
-	7	8	3	-	5	-	9				
-	9	0	3								
Total											
	115	98	73		72	51	41		83	82	46
Average											
	6.77	6.53	6.08		4.80	4.25	4.56		7.55	7.46	4.60
Average of averages											
		6.460				4.535				6.533	

Table 15. Number of offspring from first, second, and third generation mice alive at four days

	Generation 1 litters				Generation 2 litters				Generation 3 litters		
	1	2	3	4	1	2	3	4	1	2	3
						Tween 20					
	-	-	-	-	8	7	8	-	8	5	-
	-	6	7	9	5	8	8	7	6	5	8
	-	7	10	2	0	6	8	10	5	9	-
	-	-	5	-	4	5	1	-	7	8	-
	-	5	5	7	0	8	3	6	5	5	6
	-	4	4	7	1	2	9	0	6	9	-
	-	2	-	4	5	8	6	-	4	6	-
	-	7	10	3	6	7	1	5	4	8	7
	-	7	8	-	2	5	5	-	6	-	-
	-	6	3	6	7	9	1	9	4	8	-
	-	6	8	6	4	8	8	3	5	8	8
	-	7	5	8	7	8	11	-	6	6	-
	-	7	-	-	3	8	9	7			
	-	6	8	6	5	7	6	-			
					2	7	3	0			
					4	-	-	-			
					3	3	4	7			
					4	1	7	0			
Total											
		70	73	58	70	107	98	54	66	77	
Average											
		5.83	6.64	5.80	3.89	6.29	5.77	5.40	5.50	7.00	
Average of averages											
		6.090				5.337				6.250	

Table 15. (Continued)

	Generation 1 litters				Generation 2 litters				Generation 3 litters		
	1	2	3	4	1	2	3	4	1	2	3
						Tween 60					
	-	3	0	0	5	7	0	-	3	8	9
	-	5	11	2	6	4	8	5	8	5	2
	-	5	1	8	0	8	-	5	4	9	10
	-	4	6	10	0	8	10	2	1	7	0
	-	3	1	3	0	3	5	0	5	4	-
	-	6	7	1	5	9	9	8	10	10	-
	-	6	11	9	-	7	6	-	5	6	-
	-	-	10	-	3	7	8	-	4	6	6
	-	7	8	8	0	10	6	8	0	8	8
	-	9	4	0	5	7	-	-	5	7	0
	-	8	7	-	7	7	5	-			
	-	6	9	6	3	6	0	-			
	-	7	9	2							
	-	9	4	6							
	-	7	9	5							
	-	-	8	8							
Total		85	105	68	34	83	57	28	45	70	
Average		6.07	6.56	4.86	3.09	6.92	5.70	4.60	4.50	7.00	
Average of averages		5.830			5.337				5.750		

Table 15. (Continued)

Generation 1 litters				Generation 2 litters				Generation 3 litters		
1	2	3	4	1	2	3	4	1	2	3
					Control					
-	9	7	0	6	8	7	2	7	7	-
-	8	9	0	6	7	7	3	6	9	-
-	-	-	-	9	8	9	8	5	7	-
-	7	6	0	0	7	5	-	4	7	0
-	7	9	8	6	5	7	0	9	9	-
-	6	8	7	5	5	4	3	7	-	-
-	-	-	-	4	6	6	7	7	6	-
-	8	9	-	6	7	7	8	4	-	-
-	6	7	4	4	5	9	-	6	7	2
-	8	7	8	4	8	3	5	7	10	7
-	8	5	6	5	10	8	-	9	5	-
-	8	8	6	6	8	-	4			
-	8	7	7	-	7	-	-			
Total										
	83	82	46	61	91	72	40	71	67	
Average										
	7.55	7.46	4.60	5.08	7.00	6.54	4.44	6.45	7.44	
Average of averages										
		6.533			6.768				6.949	

second litter of the first generation mice. Due to conflicts in schedule, collection of these data had to be discontinued during the third breeding of the third generation mice. The third generation mice were not used in the analysis since only the second litter could be compared with the other two generations.

Since litter number had a significant effect upon the size of the litter at birth, it was not surprising to find that it had a significant effect upon the number of offspring alive at the end of 4 days (Tables 16 and 17). Since the first litter was not included in the analysis, the significance due to litter number was attributed to the smaller fourth litter. Typically the second or third litter was the largest and the fourth the smallest of the three litters used in the analysis.

When data from both the first and second generation mice were analyzed, no significant effect was indicated for treatment, generation, or for any of the interactions (Table 17). The results of the analysis involving only the first generation mice (Table 16) indicated that treatment might have a significant (P approaches .05) effect upon the number of offspring which survive to 4 days of age. The reason treatment effect approached significance was evidently due to the smaller average litter size of mice in group 6. As was pointed out in the discussion of the number of offspring

Table 16. Analysis of variance of number of offspring alive after four days, in three litters of first generation mice

Source	Degrees of freedom	Sum of squares	Mean square	F value
Litter number (L)	2	10.605665	5.302832	8.05**
Treatment (T)	6	8.200999	1.366833	2.07
L x T	12	7.768868	.647406	0.98
Error	253		.65899	

**Significant at .01.

Table 17. Analysis of variance of number of offspring alive after four days in three litters of first and second generation mice

Source	Degrees of freedom	Sum of squares	Mean square	F value
Litter number (L)	2	10.027396	5.013698	8.83**
Treatment (T)	2	.711760	.355880	0.63
L x T	4	3.032576	.758144	1.34
Generation (G)	1	.403202	.403202	0.71
L x G	2	.967975	.483988	0.85
T x G	2	.151123	.075562	0.13
L x T x G	4	.380416	.095104	0.17
Error	213		.567836	
Total	17	15.67448		

**Significant at .01.

born, this group produced smaller litters with no ready explanation as to the reason.

It is interesting to note that even though the average litter produced by group 1 was small, the average litter size at 4 days was comparable to that of any of the other groups of mice. This might be explained in part by the smaller first litter produced by group 1 mice and in part by the smaller number of mice born dead to females of group 1.

The conclusion reached from this study was that the only analyzed item which had a significant effect upon the number of offspring alive at the age of 4 days was the litter number.

Number of Offspring Weaned

The number of offspring weaned by all first generation mice is found in Table 18a. In Table 18b is recorded the number of offspring weaned by first, second, and third generation mice in which both parents were fed the same diet. These data were analyzed by the analysis of variance procedure outlined by Snedecor (1956). Summaries of the analyses are found in Tables 19 and 20.

Both analyses (Tables 19 and 20) showed litter number to have a significant effect ($P < .01$) upon the litter size at weaning. From females which weaned four litters, the

Table 18a. Number of offspring weaned from first generation mice

Group 1 20♀ x 20♂ litters				Group 2 C♀ x 20♂ litters				Group 3 20♀ x C♂ litters				Group 4 60♀ x 60♂ litters			
1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	-	-	-												
6	6	7	9	-	3	8	2	5	6	0	3	0	3	0	0
6	7	10	2	-	5	7	0	3	10	-	-	6	5	10	2
4	-	4	-	6	8	6	7	0	0	6	-	-	5	1	8
4	5	5	7	11	11	0	-	8	8	8	7	4	4	6	7
3	4	4	7	0	9	3	0	5	5	7	4	4	3	1	3
4	2	-	4	6	9	8	4	6	6	6	3	0	6	7	1
6	7	10	3	5	7	9	8	5	-	8	4	6	6	10	9
6	7	8	-	0	9	12	-	0	9	4	0	7	-	9	-
3	6	3	6	3	9	7	5	7	6	7	9	2	7	7	8
5	4	7	6	4	1	6	0	6	5	9	0	8	9	4	0
3	5	5	7	8	8	0	4	2	5	4	0	8	8	7	-
5	7	-	-	2	0	-	-	6	5	9	7	6	6	9	6
0	5	8	6	0	6	8	0	4	-	7	8	3	7	5	2
				6	8	9	0	7	10	0	0	6	9	4	6
				3	7	8	0	6	8	5	0	5	7	6	4
				2	0	5	5	-	6	0	9	6	-	8	8
Total															
55	65	71	57	56	100	96	35	70	89	80	54	71	85	94	64
Average															
3.93	5.42	6.46	5.70	4.00	6.25	6.40	2.69	4.67	6.36	5.33	3.86	4.73	6.07	5.88	4.57
Average of averages															
5.375				4.836				5.054				5.313			

Table 18a. (Continued)

Group 5 C♀ x 60♂ litters				Group 6 60♀ x C♂ litters				Group 7 C♀ x C♂ litters			
1	2	3	4	1	2	3	4	1	2	3	4
4	6	10	-	-	0	7	0	5	9	7	0
0	8	8	6	6	8	3	0	0	8	9	0
0	6	6	7	0	4	7	4	0	-	-	-
7	5	5	8	6	8	9	0	0	7	6	0
4	0	7	6	0	4	2	-	4	7	9	8
8	11	4	0	5	-	-	-	5	6	8	7
8	3	9	6	5	4	-	-	6	-	-	-
0	8	0	-	6	6	6	9	5	7	8	-
10	5	6	6	0	0	0	0	5	6	7	4
0	-	-	-	6	9	6	2	8	7	6	8
8	9	9	-	10	3	3	6	5	5	4	5
7	9	9	10	4	4	8	-	9	8	8	6
-	8	-	10	3	7	0	-	6	8	7	7
7	7	10	0	0	2	-	-				
0	9	5	0	6	7	0	-				
2	7	8	0	8	5	-	8				
5	9	0	0								
Total											
70	110	96	59	65	71	51	29	58	78	79	45
Average											
4.38	6.88	6.40	4.54	4.33	4.73	4.25	3.22	4.46	7.09	7.18	4.50
Average of averages											
5.547				4.135				5.809			

Table 18b. Number of offspring weaned from first, second, and third generation mice fed Tween 20, Tween 60, and control diets

Generation 1 litters				Generation 2 litters				Generation 3 litters		
1	2	3	4	1	2	3	4	1	2	3
					Tween 20					
0	-	-	-	8	6	7	6	8	5	7
6	6	7	9	5	8	8	6	6	5	8
6	7	10	2	0	6	1	10	5	9	4
4	-	4	-	4	5	0	-	7	7	6
4	5	5	7	0	8	0	6	5	5	6
3	4	4	7	1	2	9	0	6	9	6
4	2	-	4	5	8	5	4	4	4	8
6	7	10	3	6	7	1	5	4	8	7
6	7	8	-	2	5	5	0	6	-	0
3	6	3	6	7	9	0	9	4	8	6
5	4	7	6	4	8	8	3	5	8	8
3	5	5	7	7	8	9	-	6	6	10
5	7	-	-	3	6	8	7			
0	5	8	6	0	7	6	7			
				2	3	3	0			
				3	-	-	-			
				0	3	4	2			
				4	0	0	0			
Total										
55	65	71	57	61	99	74	64	66	74	76
Average										
3.93	5.42	6.46	5.70	3.39	5.82	4.35	4.27	5.50	6.73	6.33
Average of averages										
5.267				4.522				6.187		

Table 18b. (Continued)

Generation 1 litters				Generation 2 litters				Generation 3 litters		
1	2	3	4	1	2	3	4	1	2	3
					Tween 60					
0	3	0	0	5	7	0	-	2	2	9
6	5	10	2	6	3	7	3	7	0	6
-	5	1	8	0	6	0	5	4	2	10
4	4	6	7	0	8	8	0	1	0	0
4	3	1	3	0	3	5	0	5	4	8
0	6	7	1	5	8	8	4	8	4	7
6	6	10	9	-	7	6	-	5	6	7
7	-	9	-	3	4	8	-	4	6	6
2	7	7	8	0	10	6	8	0	8	8
8	9	4	0	5	3	-	-	5	7	0
8	8	7	-	7	7	4	-			
6	6	9	6	3	6	0	-			
3	7	5	2							
6	9	4	6							
5	7	6	4							
6	-	8	8							
Total										
71	85	94	64	34	72	52	20	41	39	61
Average										
4.73	6.07	5.88	4.57	3.09	6.00	4.73	3.33	4.10	3.90	6.10
Average of averages										
5.560				4.606				4.700		

Table 18b. (Continued)

Generation 1 litters				Generation 2 litters				Generation 3 litters		
1	2	3	4	1	2	3	4	1	2	3
				Control						
5	9	7	0	6	6	4	2	7	7	7
0	8	9	0	6	6	6	3	6	9	5
0	-	-	-	9	8	9	7	5	7	5
0	7	6	0	0	7	5	6	4	7	0
4	7	9	8	6	5	0	0	8	5	6
5	6	8	7	5	5	4	3	7	-	-
6	-	-	-	4	5	6	7	7	6	7
5	7	8	-	6	7	7	8	4	0	3
5	6	7	4	4	5	9	-	6	7	0
8	7	6	8	4	8	3	5	7	10	7
5	5	4	5	5	9	7	-	9	5	-
9	8	8	6	6	8	0	3			
6	8	7	7	0	7	0	-			
Total										
58	78	79	45	61	86	60	44	70	63	40
Average										
4.46	7.09	7.18	4.50	4.69	6.62	4.62	4.40	6.36	6.30	4.44
Average of averages										
6.243				5.307				5.703		

Table 19. Analysis of variance of number of mice weaned from the first generation females

Source	Degrees of freedom	Sums of squares	Mean square	F value
Litter No. (L)	3	22.7198	7.5733	11.49**
Treatment (T)	6	7.2325	1.2054	1.83
L x T	18	8.7046	.4836	.73
Total	27	37.022964		
Error	350		.65901	

**Significant at .01.

Table 20. Analysis of variance of number of mice weaned from the first three litters of three generations of mice fed the experimental and control diets

Source	Degrees of freedom	Sums of squares	Mean square	F value
Litter No. (L)	2	11.062871	5.531436	10.67**
Treatment (T)	2	2.859236	1.429618	2.76[f]
L x T	4	2.323430	.580858	1.12
Generations (G)	2	3.940871	1.970435	3.80*
L x G	4	6.145780	1.536445	2.96*
T x G	4	3.218031	.804508	1.55
L x T x G	8	5.460860	.682608	1.32
Total	26	35.011079		
Error	310		.51846	

**Significant at .01.

*Significant at .05.

[f]Significance approaches .05.

first and fourth litters were typically smaller than the second and third litters. Usually the second or third litter averaged the largest with a rather marked reduction in the average size of the fourth litter at weaning. These results were not surprising since there were more mice alive at 4 days in the second and third litters. Hauschka (1952) pointed out that there is a deterioration in maternal care with increasing age.

The analysis summarized in Table 20 revealed that a significant interaction existed between the litter number and generation. From data contained in Table 18, it was noted that the first litter of the third generation mice was large compared with that of the first or second generation mice, and that the third litter of second generation mice was small compared with that of the first or third generation mice. Rather wide variations may be expected in the first litter, but in the opinion of Roderick[1] one would not expect a significant variation in the third litter from one generation to another. Some, but not all of the reduction may be explained by slightly smaller litters at birth in the second generation mice fed the control and Tween 60 diets.

Treatment effect approached the .05 level of signifi-

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[1]Roderick, Thomas H., Bar Harbor, Maine. Personal letter. Private communication. 1962.

cance when the three generations of mice were compared (Table 20). The Duncan Multiple Range Test was employed to compare the means of the three treatment groups. The results of this comparison (Table 21) revealed that mice fed Tween 60 weaned significantly smaller litters than did the control mice. The same type test was used to compare the means of the seven groups of first generation mice. The results of the test (Table 22) showed that mice of group 6 (females fed Tween 60 and males fed the control diet) weaned significantly ($P < .05$) smaller litters than did mice of

Table 21. Duncan Multiple Range Test for average litter size at weaning of mice of three treatment groups over three generations[a]

(a) Shortest significant ranges

P:	(2)	(3)
R_p:	.6648	.7008

(b) Results

Treatment groups	Tween 60	Tween 20	Control
Average litter size	4.955	5.325	5.752

[a]Those means not underscored by a common line are significantly different ($P < .05$).

Table 22. Duncan Multiple Range Test for average litter size at weaning of mice in the seven treatment groups of first generation mice[a]

(a) Shortest significant ranges

P:	(2)	(3)	(4)	(5)	(6)	(7)
R_p:	1.129	1.186	1.226	1.255	1.279	1.299

(b) Results

Group	6	2	3	4	1	5	7
Treatment	60♀xC♂	C♀x20♂	20♀xC♂	60♀x60♂	20♀x20♂	C♀x60♂	C♀xC♂
	4.135	4.836	5.054	5.313	5.375	5.406	5.809

[a]Those means not underscored by a common line are significantly different (P < .05).

group 5 (females fed Tween 20 and males the control diet) or group 7 (both males and females on the control diet). It should be pointed out that mice in group 6 delivered smaller litters than did the mice in group 5 or 7. The average litter size at weaning of mice in group 4 (both males and females on the Tween 60 diet) was not significantly different from that of mice in any other group. Even though the results are not clear-cut in every case, there is a suggestion that females fed Tween 60 diet tend to wean smaller litters than do the mice on the control diet.

The treatment by generation interaction was not statistically significant at the .05 level, but an interesting observation was made relative to performance of the Tween 20 mice. The third generation mice fed Tween 20 weaned litters which averaged 6.19 mice compared to the 4.52 mice per litter weaned by the second generation mice on the Tween 20 diet. This is somewhat surprising since the average litter sizes at birth were 6.90 and 6.59 respectively. There was no apparent reason why the third generation mice should keep so many more of their offspring than did the second generation mice. There was no such variation in the mice on either the control or Tween 60 diet.

Weight of Offspring at Weaning

The weight of offspring weaned by all first generation mice is recorded in Table 23. In Table 24 is recorded the weight of offspring weaned by first, second, and third generation mice in which both parents were fed the same diet. These data were analyzed by the analysis of covariance procedure as outlined by Snedecor (1956). The summaries of these analyses are recorded in Tables 25, 26, 27, and 28. The covariant in the analyses was the number of mice in the litter at weaning, which means that variations in weaning weight caused by litter size at weaning were statistically

Table 23. Weaning weight of offspring for first generation mice

Group 1 20♀ x 20♂ litters				Group 2 C♀ x 20♂ litters				Group 3 20♀ x C♂ litters			
1	2	3	4	1	2	3	4	1	2	3	4
0	-	-	-								
-	-	69(7)	79(9)	-	-	84(8)	20(2)	-	-	-	20(3)
-	65(7)	68(10)	9(2)	-	45(5)	71(7)	0	-	-	-	-
-	-	38(4)	-	-	75(8)	67(6)	59(7)	-	0	41(6)	-
-	-	43(5)	59(7)	-	76(11)	0	-	-	73(8)	76(8)	67(7)
-	-	39(4)	49(7)	-	88(9)	39(3)	0	-	40(5)	56(7)	36(4)
-	-	-	40(4)	-	81(9)	80(8)	48(4)	-	56(6)	56(6)	27(3)
-	59(7)	74(10)	26(3)	-	58(7)	85(9)	64(8)	-	-	73(8)	48(4)
-	53(7)	50(8)	-	-	77(9)	83(12)	-	-	48(9)	41(4)	0
-	59(6)	31(3)	54(6)	-	88(9)	68(7)	50(5)	-	58(6)	70(7)	72(9)
-	20(4)	35(7)	38(6)	-	5(1)	59(6)	0	-	45(5)	70(9)	0
-	30(5)	39(5)	57(7)	-	81(8)	0	48(4)	-	-	39(4)	0
-	45(7)	-	-	-	-	-	-	-	46(5)	64(9)	53(7)
-	39(5)	64(8)	44(6)	-	65(6)	79(8)	0	-	-	48(7)	45(8)
				-	78(8)	91(9)	0	-	55(10)	0	0
				-	75(7)	65(8)	0	-	62(8)	41(5)	0
				-	0	57(5)	47(5)	-	67(6)	-	66(9)
Totals											
-	370(48)	550(71)	455(57)	-	892(97)	928(96)	336(35)	-	550(68)	675(80)	434(54)
Total											
1375(176)				2156(228)				1659(202)			

Table 23. (Continued)

Group 4 60♀ x 60♂ litters				Group 5 C♀ x 60♂ litters				Group 6 60♀ x C♂ litters			
1	2	3	4	1	2	3	4	1	2	3	4
-	32(3)	0	0	-	-	95(10)	-	-	0	63(7)	0
-	-	70(10)	18(2)	-	85(8)	70(8)	35(6)	-	65(8)	24(3)	0
-	-	5(1)	47(8)	-	-	61(6)	69(7)	-	-	57(7)	-
-	-	57(6)	59(7)	-	-	56(5)	86(8)	-	68(8)	66(9)	0
-	-	10(1)	27(3)	-	0	73(7)	67(6)	-	45(4)	22(2)	-
-	-	62(7)	6(1)	-	99(11)	52(4)	0	-	-	-	-
-	-	72(10)	49(9)	-	-	79(9)	55(6)	-	49(4)	-	-
-	-	58(9)	-	-	90(8)	0	-	-	52(6)	60(6)	73(9)
-	60(7)	55(7)	46(8)	-	62(5)	70(6)	62(6)	-	0	-	0
-	75(9)	34(4)	0	-	-	-	-	-	68(9)	36(6)	12(2)
-	71(8)	52(7)	-	-	73(9)	69(9)	-	-	37(3)	30(3)	42(6)
-	63(6)	56(9)	55(6)	-	-	85(9)	57(10)	-	38(4)	55(8)	-
-	57(7)	33(5)	14(2)	-	73(8)	-	74(10)	-	55(7)	0	-
-	76(9)	42(4)	55(6)	-	63(7)	82(10)	0	-	20(2)	0	-
-	58(7)	33(6)	28(4)	-	80(9)	47(5)	0	-	68(7)	0	-
-	-	61(8)	60(8)	-	73(7)	64(8)	0	-	55(5)	-	52(8)
				-	94(9)	0	0				
Totals											
-	492(56)	700(94)	464(64)	-	792(81)	903(96)	505(59)	-	620(67)	413(51)	179(25)
Total											
		1656(214)				2200(236)				1212(143)	

Table 23. (Continued)

		Group 7 C♀ x C♂ litters	
1	2	3	4
-	80(9)	75(7)	0
-	-	81(9)	0
-	-	-	-
-	73(7)	64(6)	0
-	67(7)	81(9)	76(8)
-	58(6)	69(8)	58(7)
-	-	-	-
-	64(7)	68(8)	-
-	-	-	-
-	65(6)	65(7)	55(4)
-	63(7)	56(6)	77(8)
-	39(5)	39(4)	45(5)
-	75(8)	71(8)	58(6)
-	73(8)	73(7)	67(7)
Totals			
-	657(70)	742(79)	436(45)
Total			
		1835(194)	

Table 24. Weaning weight of offspring for first, second, and third generation mice fed Tween 20, Tween 60, and control diets

Tween 20										
Generation 1 litters				Generation 2 litters				Generation 3 litters		
1	2	3	4	1	2	3	4	1	2	3
-	-	-	-	60(8)	51(6)	54(7)	54(6)	55(8)	43(5)	68(7)
-	-	69(7)	79(9)	42(5)	54(8)	60(8)	58(6)	46(6)	40(5)	65(8)
-	65(7)	68(10)	9(2)	0	46(6)	4(1)	80(10)	41(5)	62(9)	42(4)
-	-	38(4)	-	32(4)	43(5)	0	-	56(7)	47(7)	44(6)
-	-	43(5)	59(7)	0	58(8)	0	48(6)	37(5)	37(5)	60(6)
-	-	39(4)	49(7)	7(1)	18(2)	73(9)	0	48(6)	62(9)	60(6)
-	-	-	40(4)	43(5)	61(8)	48(5)	43(4)	32(4)	32(4)	56(8)
-	59(7)	74(10)	26(3)	48(6)	62(7)	35(1)	54(5)	24(4)	55(8)	60(7)
-	53(7)	50(8)	-	19(2)	45(5)	43(5)	0	38(6)	-	0
-	59(6)	31(3)	54(6)	53(7)	66(9)	0	65(9)	35(4)	65(8)	65(6)
-	20(4)	35(7)	38(6)	40(4)	56(8)	54(8)	38(3)	40(5)	64(8)	74(8)
-	30(5)	39(5)	57(7)	44(7)	56(8)	58(9)	-	48(6)	52(6)	89(10)
	45(7)	-	-	24(3)	28(6)	40(8)	56(7)			
-	39(5)	64(8)	44(6)	0	42(7)	53(6)	61(7)			
				20(2)	18(3)	25(3)	0			
				20(3)	-	-	-			
				-	23(3)	37(4)	-			
				27(4)	-	-	12(2)			
Totals										
-	370(48)	550(71)	455(57)	497(61)	727(99)	552(74)	569(65)	500(66)	559(74)	683(76)
Total										
1375(176)				2345(299)				1742(216)		

Table 24. (Continued)

Tween 60										
Generation 1 litters				Generation 2 litters				Generation 3 litters		
1	2	3	4	1	2	3	4	1	2	3
-	32(3)	0	0	47(5)	50(7)	0	-	15(2)	11(2)	70(9)
-	-	70(10)	18(2)	55(6)	29(3)	63(7)	27(3)	48(7)	0	45(6)
-	-	5(1)	47(8)	0	35(6)	0	45(5)	34(4)	-	78(10)
-	-	57(6)	59(7)	0	62(8)	46(8)	0	6(1)	0	0
-	-	10(1)	27(3)	0	30(3)	38(5)	0	35(5)	42(4)	71(8)
-	-	62(7)	6(1)	30(5)	60(8)	52(8)	22(4)	48(8)	32(4)	45(7)
-	-	72(10)	49(9)	-	55(7)	45(6)	-	40(5)	43(6)	57(7)
-	-	58(9)	-	26(3)	28(4)	54(8)	-	30(4)	58(6)	69(6)
-	60(7)	55(7)	46(8)	0	57(10)	47(6)	61(8)	0	57(8)	60(8)
-	75(9)	34(4)	0	48(5)	16(3)	-	-	36(5)	51(7)	0
-	71(8)	52(7)	-	50(7)	49(7)	-	-			
-	63(6)	56(9)	55(6)	30(3)	40(6)	-	-			
-	57(7)	33(5)	14(2)							
-	76(9)	42(4)	55(6)							
-	58(7)	33(6)	28(4)							
-	-	61(8)	60(8)							
-	-	-	-							
Totals										
-	492(56)	700(94)	464(64)	286(34)	511(72)	345(48)	155(20)	292(41)	294(37)	417(57)
Total										
1656(214)				1297(174)				1003(129)		

Table 24. (Continued)

Control										
Generation 1 litters				Generation 2 litters				Generation 3 litters		
1	2	3	4	1	2	3	4	1	2	3
	80(9)	75(7)	0	53(6)	56(6)	33(4)	12(2)	58(7)	74(7)	68(7)
	-	81(9)	0	58(6)	51(6)	54(6)	26(3)	51(6)	93(9)	56(5)
	-	-	-	74(9)	78(8)	85(9)	79(7)	49(5)	67(7)	52(5)
	73(7)	64(6)	0	0	71(7)	33(5)	44(6)	38(4)	-	0
	67(7)	81(9)	76(8)	59(6)	54(5)	0	0	74(8)	47(5)	59(6)
	58(6)	69(8)	58(7)	49(5)	53(5)	31(4)	30(3)	55(7)	-	-
	-	-	-	34(4)	51(5)	63(6)	69(7)	57(7)	63(6)	63(7)
	64(7)	68(8)	-	53(6)	68(7)	58(7)	81(8)	42(4)	0	34(3)
	65(6)	65(7)	55(4)	39(4)	51(5)	-	-	53(6)	65(7)	0
	63(7)	56(6)	77(8)	35(4)	75(8)	34(3)	61(5)	65(7)	93(10)	65(7)
	39(5)	39(4)	45(5)	43(5)	84(9)	62(7)	-	78(9)	48(5)	-
	75(8)	71(8)	58(6)	64(6)	85(8)	0	32(3)			
	73(8)	73(7)	67(7)	-	65(7)	-	-			
Totals										
	657(70)	742(79)	436(45)	561(61)	842(86)	453(51)	353(36)	620(70)	550(56)	397(40)
Total										
	1835(194)			2209(234)				1567(166)		

Table 25. Analysis of covariance of weaning weight of first generation offspring in litters 2 through 4

Sources (adjusted)	Degrees of freedom	Sum of squares	Mean square	F value
Litter No. (L)	2	110.2809	55.1405	7.51**
Treatment (T)	6	629.11	104.8519	14.28**
L x T	12	137.9115	11.493	1.56
Error	190	139.7336	7.3407	

**Significant at .01.

Table 26. Analysis of covariance of weaning weights of first and second generation offspring in litters 2 through 4

Sources (adjusted)	Degrees of freedom	Sum of squares	Mean square	F value
Litter No. (L)	2	21.0994	10.5497	1.48
Treatment (T)	2	526.0389	263.0194	36.9**
Generation (G)	1	21.1946	21.1946	2.98
L x T	4	45.2712	11.3178	1.59
G x T	2	12.8422	6.4211	.90
G x L	2	17.7432	8.8716	1.25
G x T x L	4	65.7506	16.4377	2.31
Error	167	1189.0086	7.1198	

**Significant at .01.

Table 27. Analysis of covariance of weaning weights of second and third generation mice in litters 1 through 3

Sources (adjusted)	Degrees of freedom	Sum of squares	Mean square	F value
Litter No. (L)	2	28.4770	14.2385	2.83
Treatment (T)	2	460.2741	230.1371	45.7**
Generation (G)	1	36.8717	36.8717	7.33**
L x T	4	76.7196	19.1799	3.81**
G x T	2	5.6099	2.8050	0.56
G x L	2	83.5785	41.7892	8.31**
G x T x L	4	17.7486	4.4372	.88
Error	167	840.2353	5.0313	

**Significant at .01.

Table 28. Analysis of covariance of weaning weights of first, second, and third generation mice in litters 2 and 3

Sources (adjusted)	Degrees of freedom	Sum of squares	Mean square	F value
Litter No. (L)	1	1.0687	1.0687	0.17
Treatment (T)	2	524.5069	262.2534	42.9**
Generation (G)	2	93.5481	46.7740	7.65**
L x T	2	38.9189	19.4595	3.18*
G x T	4	21.4715	5.3678	0.88
G x L	2	39.0514	19.0514	3.19*
G x T x L	4	66.5595	16.6399	2.72*
Error	166	1014.6183	6.1122	

**Significant at .01.

*Significant at .05.

removed from the analyses. By adjusting the litters to a common size, any effect on the weaning weights due to litter size was eliminated. The adjustment of litter size accounted for the major portion of variation due to litter number. The litter number proved to have a significant effect ($P < .01$) on the weaning weight of offspring from only first generation mice. After the litters were adjusted to a common size (Table 29), the second litter was heaviest and the fourth litter the lightest.

Interpretation of any factor which affects the covariant (litter size at weaning) needs to be made with care. There would then be certain hazards in attaching significance to interpretation which involves litter number since there is an interaction between this and litter size. The F resulting from the ratio of litter number to error was significant in the first generation (Table 25) when litter size was the controlled covariant.

In order to get as complete analysis as possible while maintaining balance, analyses were made comparing the weaning weights of the 7 groups of first generation mice for litters 2 through 4 (Table 25); first and second generation mice for litters 2 through 4 (Table 26); second and third generation mice for litters 1 through 3 (Table 27); and the first, second and third generation mice for litters 2 and 3 (Table 28). Treatment proved to be highly significant ($P < .01$) in

Table 29. Comparison of adjusted litter weights (generation X litter)

	Generation 1 (Groups 1, 4, and 7)	Generation 1 (All groups)	Generation 2	Generation 3
Litter 1	no data		49.280	47.102
Litter 2	53.550	55.663	49.293	51.757
Litter 3	50.648	53.570	47.359	55.416
Litter 4	50.133	50.132	51.209	no data
Total	51.444		49.217	51.423

each of the analyses.

Information found in Table 30 revealed that when litters were adjusted to a common size the mice on experimental diets consistently weaned litters which were several grams lighter in weight than those weaned by control mice.

Check of first generation mice (Table 31) revealed that the diet of males had very little, if any, effect on the weaning weights of the offspring. In groups 3 and 6, in which females were fed an experimental diet and males fed the control diet, the weaning weight of the litters was near that of groups 1 and 4 in which both parents were fed an experimental diet. In groups 2 and 5 in which the females were fed the control diet and males fed an experimental

Table 30. Comparison of adjusted litter weights (treatment X litter)

	Treatment		
	Tween 20	Tween 60	Control
Litter 1	45.142	45.317	53.111
Litter 2	45.619	48.198	60.787
Litter 3	49.379	46.696	57.051
Litter 4	50.04	44.340	57.643
Total	47.5346	46.400	57.701

Table 31. Adjusted litter weights of first generation mice

Group number	Treatment	Weight
1	(20♀ x 20♂)	47.039
2	(C♀ x 20♂)	58.733
3	(20♀ x C♂)	50.062
4	(60♀ x 60♂)	47.759
5	(C♀ x 60♂)	60.033
6	(60♀ x C♂)	48.671
7	(C♀ x C♂)	59.537

diet, the litters were comparable in weight to those of group 7 in which both parents were fed the control diet.

As was pointed out previously there are hazards in attaching significance to interpretations of interactions involving litter number that show a significant F because the litter number and the covariant (litter size at weaning) are related. However, certain of these should be considered.

Analyses recorded in Tables 27 and 28 indicated an interaction between litter number and treatment. Information found in Table 30 revealed that litters 3 and 4 of mice on Tween 20 were heavier than others in the experimental groups and litter 1 of the control mice was several grams lighter in weight than would be expected on the basis of the weight of other litters of control mice. The litters which appeared to deviate most from the average were from third generation mice. The adjusted weights of litters from third generation mice are recorded in Table 32. The lighter average weight of the first litter of control mice was apparently due to the third generation control mice of which there were 5 litters whose adjusted weight was less than 52 grams each, while there were only 3 litters whose adjusted weight was near the more normal 58 grams. In litter 3 of the third generation mice on Tween 20 diet (Table 32), there were 5 litters in which the adjusted weight was over 60 grams while there were only 3 litters whose adjusted weight was less

Table 32. Adjusted weights of litters from third generation mice

Mouse	Tween 20			Tween 60			Control		
	Litter 1	Litter 2	Litter 3	Litter 1	Litter 2	Litter 3	Litter 1	Litter 2	Litter 3
1	42.93	49.73	61.53	41.53	37.53	50.33	51.53	67.53	61.53
2	46.13	46.73	51.93	41.53		45.13	51.13	73.33	62.73
3	47.73	42.33	55.33	47.32	-	51.73	55.73	60.53	58.73
4	49.53	40.53	44.13	39.13			51.33	-	
5	43.73	43.73	60.13	41.73	55.33	57.93	60.93	53.47	59.13
6	48.13	42.33	60.13	34.93	45.33	38.53	48.53	-	-
7	45.33	45.33	42.93	46.73	43.13	50.53	50.53	63.13	56.53
8	37.33	41.93	53.53	43.33	58.13	69.13	55.33		53.93
9	38.13	-			43.93	47.93	53.13	58.53	
10	48.33	51.93	65.13	42.73	44.53	47.93	58.54	66.73	58.53
11	46.73	50.93	60.33				58.33	54.73	-
12	48.13	52.13	33.73						

than 51 grams. This same trend was evident in the fourth litter of the second generation mice fed the Tween 20 diet. There was no apparent explanation why there should have been more heavy litters and fewer light litters in these cases.

Interaction between generation and litter number was indicated by information contained in Tables 27 and 28. The interaction was likely due in large part to litters of the third generation mice. A comparison of adjusted weights of litters for the 3 generations of mice (Table 29) showed that litter 1 of the third generation was the lightest and that litter 3 of the third generation mice was the heaviest of all litters. As indicated in Table 32 only one litter from the first breeding of third generation control mice had an adjusted weight as great as 60 grams. There was only one litter from the first breeding of third generation mice fed Tween 60 or Tween 20 whose adjusted weight was as much as 49.5 grams. In litter 3 of the experimental mice there were 13 litters whose adjusted weight was over 50 grams. There was one litter from the third breeding of control mice whose adjusted weight was less than 56 grams. The above facts account, at least in part, for the interaction between generation and litter number. From the data available, it appears that the third generation mice, considered jointly, tend to wean litters which become progressively heavier.

Three way interaction involving generation, treatment and litter number was significant ($P < .05$) only when litters 2 and 3 of the first, second and third generations were considered.

Fifth Breeding

Some of the first generation females weaned their fourth litter early enough to permit a fifth breeding during the period covered by this study. As was indicated in the introduction, some of these mice were entering the age at which an age effect upon reproductive functions could be expected (Bruce, 1947). This, coupled with the fact that the number of mice involved in the fifth breeding was much reduced, made it undesirable to include these data in the general body of analyzed data.

The results of the fifth breeding are included in Table 33. One of the most noticeable differences was the increased number of offspring born dead in many of the groups. This is in conformity with data reported by Little and Pearsons (1940). As indicated in previous discussion, the diet of the female had the greatest effect upon the offspring. Considering this, along with the relatively small number of mice involved, it was thought that a clearer presentation

Table 33. Results of the fifth breeding of some first generation mice

Mouse number	Number born	Number born dead	Number alive at 4 days	Number at weaning	Litter weight at weaning
			20♀ x 20♂		
6	10	0	-	6	46
7	4	0	-	2	18
8	4	0	4	4	37
9	6	0	6	6	54
10	3	1	-	0	-
13	10	0	10	5	26
Totals	37	1	20	23	181
			20♀ x C♂		
1	4	0	0	0	-
4	delivered but found no offspring				
5	7	0	7	6	-
7	no pregnancy in 1 1/2 months				
10	10	1	0	0	-
17	8	4	0	0	-
Totals	35	11	7	6	-
			60♀ x 60♂		
1	found 1 dead and 2 fragments				
2	6	1	0	0	-
3	9	0	6	6	34
4	8	3	2	0	-
5	9	2	0	0	-
7	7	4	0	0	-

Table 33. (Continued)

Mouse number	Number born	Number born dead	Number alive at 4 days	Number at weaning	Litter weight at weaning
12	7	0	7	5	36
16	delivered but found no offspring				
Totals	46	10	15	11	70
			60♀ x C♂		
1	no pregnancy in 1 month				
2	6	0	6	6	50
3	1	0	1	0	-
8	5	1	4	2	20
9	6	0	0	0	-
12	3	0	2	0	-
13	9	1	0	0	-
Totals	30	2	13	8	70
			C♀ x 20♂		
1	found parts of 3				
2	8	0	8	8	70
3	5	1	0	0	-
4	6	0	6	5	50
8	6	0	5	5	40
9	6	5	0	0	-
12	10	10	0	0	-
Totals	41	16	19	18	160

Table 33. (Continued)

Mouse number	Number born	Number born dead	Number alive at 4 days	Number at weaning	Litter weight at weaning
			C♀ x 60♂		
1	5	0	5	5	56
3	3	3	0	0	-
4	8	2	4	3	-
5	6	2	4	2	19
7	9	9	0	0	-
9	5	5	0	0	-
Totals	36	21	13	10	65
			C♀ x C♂		
2	did not become pregnant in 1 month				
4	may have delivered but found no young				
5	8	1	7	7	64
6	3	1	0	0	-
Totals	11	2	7	7	64

could be made if all groups which had females on the same diet were combined for this discussion.

There were 11 litters produced by the Tween 20 females, 12 litters by the Tween 60 females and 14 litters by the females on the control diet. The average litter size at birth was about the same in each of the 3 groups. The

Tween 20 mice averaged 6.55 mice per litter, the Tween 60 mice averaged 6.33 mice and the controls averaged 6.29 mice per litter.

There were 12 of 72 offspring born dead in the Tween 20 group, 12 of 76 born dead in the Tween 60 group and 39 of 88 born dead in the control mice. There was a higher percentage of offspring born dead in the control mice, but a greater number of mice died between birth and weaning in the experimental groups. Twenty-eight of the offspring of mice fed the Tween 20, 45 of those fed Tween 60, and 17 in the control group died between birth and weaning. At weaning there were 30, 19 and 35 young remaining in the respective groups.

As would be expected on the basis of the findings discussed in the previous section, the average weaning weight of the offspring from the control mice was greater than that of offspring from the experimental mice. The average weights were: 7.87 grams for ones on Tween 20, 7.39 grams for those of the Tween 60 group and 9.03 grams for offspring of the control mice.

Rate of Weight Gain Before Weaning

As was noted in the discussion of weaning weights of the various groups of mice (see section on "Weight of Offspring at Weaning"), the average weaning weight of the offspring of mice fed Tween 60 or Tween 20 was significantly less than that of offspring of mice on the control diet. Near the end of the experimental period a subsidiary study was made to determine when, in the nursing period, this difference occurred.

Litter weights of mice used in this study were taken at ages 4 days, 14 days, and 21 days. The average weight per mouse was determined by dividing the total litter weight by the number of mice in the litter. The number of mice in the litter, the weight of the litter at 4, 14, and 21 days, the average gain per mouse between the ages of 4 and 14 days and between the ages of 14 and 21 days, are recorded in Tables 34, 35, and 36. For convenience in comparing the weaning weights of the sample of mice used in this study with that of the total experimental and control mice, the totals from Table 24 are recorded in Table 37. To make as many observations as possible, all mice which were less than two weeks of age at the beginning of this study were included. There are, thus, more entries of litter weight at 14 and 21 days of age than at 4 days.

Even though some generation effect upon weaning weight of offspring was indicated in Tables 27 and 28, a comparison between the average weaning weights as recorded in Table 37 revealed that the average weaning weight of the mice on a specific diet did not vary more than .33 grams from one

Table 34. Weights and average gain in grams of young C57BL/6 mice on Tween 20 diet

Female parent	4 days		14 days		Aver. gain per mouse 4-14 days	21 days		Aver. gain per mouse between 14-21 days
	No.	Wt.	No.	Wt.		No.	Wt.	
1G'-4	4	12.5	4	26	3.38	4	37	2.75
2E'-3	10	21.0	10	38	1.70	5	26	1.40
2P'-4	7	14.0	7	46	4.67	7	53	0.90
2X'-5'-3	10	23.0	10	63	4.00	10	80	1.70
1F'-1'-2	7	13	6	37	4.31	6	48	1.83
1G'-3'-1	9	19	9	54	3.89	9	73	2.11
1G'-3'-2	N.W.[a]		5	37	-[b]	5	48	2.20
1G'-4'-1	N.W.		7	45	-	5	43	2.17
2E'-5'-1	9	18	8	40	3.00	8	40	0.0
2E'-5'-2	6	13	6	41	4.66	6	53	2.00
2E'-5'-3	3	6	3	22	5.33	3	27	1.67
1H'-4'-2	4	12	4	28	4.00	4	37	2.25
1S'-1'-1	8	11	2	8	2.62	0	-	-
2X'-5'-1'-1	5	11	5	37	5.20	5	43	1.20
2X'-5'-1'-2	5	12	5	33.5	4.30	5	40	1.30
2X'-5'-1'-3	9	22	9	53	3.56	9	62	1.00
1S'-1'-1'-1	8	19	7	37	2.91	7	47	1.42
1G'-4'-1'-1	5	13	5	26	2.60	5	37	2.20
1N'-3'-1'-1	9	18	9	50	3.55	9	62	1.34
2S'-3'-2'-1	6	14	5	26	2.87	4	32	2.80
2S'-3'-2'-2	8	19	8	41	2.76	8	55	1.74
1G'-3'-2'-1	8	22	8	53	3.89	8	65	1.48
2X'-5'-2'-1	8	24	8	60	4.50	8	64	0.50
2X'-5'-2'-2	6	15	N.W.		-	6	52	-
Total	154	351.5	150	901.5	77.70	146	1124	35.96
Average	2.28		6.01		3.70	7.70		1.63

[a]N.W.: not weighed.

[b] - : no entry possible.

Table 35. Weights and average gain in grams of young C57BL/6 mice on Tween 60 diet

Female parent	4 days No.	4 days Wt.	14 days No.	14 days Wt.	Aver. gain per mouse 4-14 days	21 days No.	21 days Wt.	Aver. gain per mouse between 14-21 days
1C'-1	7	21	7	48	3.86	5	36	0.34
1C'-1'-1	6	16	6	43	4.50	6	45	0.33
1C'-1'-2	8	23	8	49	3.25	8	54	0.62
1C'-1'-3	6	16	6	39	3.83	6	47	1.33
1G'-6'-1'-1	9	21	2	11.5	3.42	were not weaned at proper time		
1G'-6'-1'-3	4	14	4	35	5.25	4	42	1.75
1C'-1'-2'-1	N.W.[a]		5	33	--[b]	5	40	1.40
2G'-3'-1'-2	N.W.		5	33	--	5	35	0.40
1C'-1'-4'-1	N.W.		6	60	--	6	70	1.70
1G'-6'-2'-1	8	20	6	41	4.33	2	11	-1.33[c]
Total	48	131	55	392.5	28.44	47	380	27.87
Average	2.73		7.14		4.06	8.09		0.99

[a]N.W.: not weighed.

[b] -- : no entry possible.

[c]Because this reaction is very atypical, and cannot be accounted for, it was not used in calculating the average weight gain for mice between ages 14 and 21 days.

Table 36. Weights and average gain in grams of young C57BL/6 mice on control diet

Female parent	4 days No.	4 days Wt.	14 days No.	14 days Wt.	Aver. gain per mouse 4-14 days	21 days No.	21 days Wt.	Aver. gain per mouse between 14-21 days
2F'-1	6	17	5	35	4.17	5	50	3.00
1K'-1	5	12	5	27	3.00	5	40	2.60
1G'-2	2	4	2	13	4.50	2	19	3.00
1I'-2	12	19	11	48	2.82	10	57	1.30
1K'-3	7	13	7	43	4.28	7	64	3.00
2D'-2'-3	N.W.[a]		6	37	--[b]	6	54	2.83
1G'-1'-1	N.W.		6	40	--	6	56	2.66
2P'-1'-1	5	10	4	26	4.50	4	39	3.25
2P'-1'-2	N.W.		9	58	--	9	85	3.11
2P'-1'-3	5	10	5	25	3.00	5	33	1.60
			(2 days over)					
2P'-1'-4	7	15	7	32	2.43	0	--	--
2D'-2²-2	N.W.		6	46	--	5	51	2.53
2D'-2²-4	10	27	9	52	3.08	9	84	3.55
2D'-2'-3'-1	7	19	7	50	4.43	7	74	3.43
1G'-1'-1'-1	9	21	9	59	4.23	9	93	3.77
1G'-1'-1'-2	7	18	7	48	4.29	7	67	2.71
2D'-2'-1'-2	6	15	6	47	5.33	6	63	2.67
2P'-1'-2'-3	N.W.		9	55	--	9	78	2.56
Total	88	200	120	741	50.06	111	1007	47.57
Average	2.27		6.18		3.85	9.07		2.80

[a]N.W.: not weighed.

[b] -- : no entry possible.

Table 37. Comparison of weaning weights from three generations of experimental and control C57BL/6 mice

	First generation			Second generation			Third generation		
Diet	No. weaned	Total wt.	Ave. wt.	No. weaned	Total wt.	Ave. wt.	No. weaned	Total wt.	Ave. wt.
Tween 20	176	1,375	7.81	299	2,345	7.84	216	1,742	8.06
Tween 60	214	1,656	7.75	174	1,297	7.45	129	1,003	7.78
Control	194	1,835	9.46	234	2,209	9.49	166	1,567	9.44

generation to another. Since there was not a great difference in the average weaning weight of mice with the same dietary history in one generation as compared with that of another, mice from all 3 generations were included in this subsidiary study. The average weaning weights of offspring used in this study are comparable to the average weight of all offspring in the representative dietary group.

There was no great difference in the average weight gain of any of the mice between the ages of 4 and 14 days as revealed in Tables 34, 35, and 36. The average weight gain between the ages of 4 and 14 days was 3.70 grams, 4.06 grams and 3.85 grams, respectively, for mice whose mothers were on the Tween 20, Tween 60, and control diets. A comparison of the weight gain of mice between the ages of 14 and 21 days revealed a significant difference. The mice whose mothers were on the diet containing Tween 20 made an average gain of 1.63 grams; those on Tween 60 gained an average of 0.99 grams while the control mice had an average gain of 2.80 grams. The average weight of mice of each litter was subjected to an analysis of variance. This analysis showed significance beyond the .01 level. On the basis of the information obtained from the analysis, it is quite evident that the mice whose mothers were on the control diet made a greater weight gain between the ages of 14 and 21 days than the mice whose mothers were fed diets containing 10% Tween 20 or Tween 60. This difference in weight gain may be related to the fact that mice begin eating solid food when they reach about 14 days of age. The results of this study have raised several questions which can only be answered by further investigation.

Is the difference in weight gain related to taking solid foods containing the additive? As was noted in the discussion, the difference in rate of gain occurred about the time the offspring reached two weeks of age. This is the age young mice start taking solid foods. It is possible that the mice did not take as much food with the additive as they would have the control because of difference in taste. As was noted by Harris *et al.* (1951) in hamsters, Tween compounds can irritate the intestine. As a result, it could interfere with digestion and absorption of food. It was also noted that mice on the experimental diets frequently had diarrhea. It is possible that the <u>Tween</u> compounds upset the functioning of the digestive system and thus interfered with food utilization. It is also possible that these test substances upset the flora of the intestine to such an extent that it interfered with the normal functioning of the digestive tract. Bourke and Fitzhugh (1953) noted that the intestinal flora of rats was altered by 5% and 25% levels of polyoxyethylene sorbiten esters of lauric and stearic acids.

Does the mouse have to make some special type of adjustment to diets containing the <u>Tween</u> preparations? As was noted previously there is a definite difference in the average weaning weight of mice fed control diets as compared with those fed the experimental diets. It will be noted in the following section that there was no significant difference in the adult weight of the mice. It appears that mice are able to adjust to the experimental diet and then "catch up" with those on the control diet.

If an adjustment does occur, does it have anything to do with the size of the intestine? It was rather common to find animals with an enlarged or bloated ceca. Gas was often present. Enlarged cecae in hamsters were noted by Eagle and Poling (1956). It is possible that this enlargement is in some way related to the adjustment of the animal which enables it to utilize the food containing a Tween compound.

Is Tween 60 or Tween 20 found in the milk of lactating females fed the respective diets? If Tween causes retardation in weight gain, it would seem that the Tweens would not be getting into the milk in very large quantities because suckling mice of females fed the experimental diets gained at comparable rates to those of control mice. It is possible that the Tween would have been altered enough by the time it reached the milk that it would not cause undesirable results. Another possibility is that results of taking Tweens is related to some degree to the type of food with which the Tween is mixed. Ershoff and Hernandez (1959) and Allison *et al*. (1952) reported that bulky foods as alfalfa meal reduced toxic effects of feeding certain surface active agents.

Weight of Adult Mice

Many of the first and second generation control and experimental mice were weighed and killed after they had produced the desired number of litters. The average age of the mice at the time they were killed was about 10.5 months. The individual weights of these mice are recorded in Table 38. There were 35 females on Tween 20, 35 females on Tween 60, and 48 females on the control diet included in this section of the investigation. The average weights of these

Table 38. Weights of adult experimental and control mice (C57BL/6)

Tween 20		Tween 60		Control	
Females	Males	Females	Males	Females	Males
30	33	32	33	34	33
35	35	37	32	30	34
28	34	25	33	30	34
29	31	29	30	32	38
21	33	31	31	31	33
29	31	28	35	34	32
29	33	35	31	29	28
30	32	27	33	39	31
34	32	34	37	26	33
28	34	30	35	27	31
32	31	33	35	30	31
30	32	29	35	35	31
35	30	31	35	29	30
32	32	35	35	30	30
30	30	31	33	30	33
34	32	35	35	33	31
30	36	28	27	28	28
22	32	30	35	30	33
26	35	29		33	36
32	33	29		33	33
30	33	27		32	
32	38	19		31	
36	37	30		30	
33		32		27	
30		30		29	
31		31		30	
34		35		29	

Table 38. (Continued)

Tween 20		Tween 60		Control	
Females	Males	Females	Males	Females	Males
32		33		33	
33		32		30	
33		28		31	
44		30		38	
26		33		31	
23		32		32	
32		24		30	
28		32		37	
				29	
				28	
				35	
				33	
				31	
				28	
				34	
				32	
				28	
				32	
				27	
				27	
				33	
Total					
1,073	759	1,066	590	1,490	643
Average					
30.67	32.13	30.46	32.78	31.04	32.15

mice were 30.67 grams, 30.46 grams, and 31.04 grams, respectively. There was a variation of 12 to 15 grams in weight of individual mice within a dietary group, but the greatest difference in the average weight of mice between groups was only 0.58 grams.

The sample of weighed males included 23 on Tween 20 diet, 18 on Tween 60 diet and 20 on the control diet. These had average weights of 32.13 grams, 32.78 grams, and 32.15 grams, respectively. As was true of the females, there was more variation (8-11 grams) among the individuals within a dietary group than between the average weights of groups.

From the information obtained in this study, it is evident that the feeding of Tween 20 or Tween 60 at a 10% concentration had no significant effect upon the body weight of first or second generation C57BL/6J strain mice, about 10.5 months of age, as compared to the weight of comparable control mice.

The average weights of C57 female mice recorded by Fenton and Cowgill (1948) are approximately the same as those recorded in Table 38 of this report. The weights of the males included in the study are very near the 32 grams reported by Silberberg and Silberberg (1960) for C57BL/6 control mice 12 months of age. Silberberg and Silberberg (1954) reported that C57BL males fed a stock diet reached their maximum weight of 32.3 grams at the age of 15 months.

Ovary Weights

Ovary weights were taken from a sample of first generation mice approximately 10.5 months of age. These weights are recorded in Table 39. No attempt was made to obtain ovaries at any specific time in the estrus cycle. Of the 125 ovaries weighed, only 10 appeared to be completely inactive. Five of these came from mice on the Tween 20 diet and 5 came from the control group. None of those from the Tween 60 group appeared completely inactive, but because the Tween 60 sample was the smallest, it is doubtful if any significance could be attached to the absence of inactive ovaries in this group.

The mice used in this portion of the study included 19 on the Tween 20 diet, 15 on the Tween 60 diet, and 29 mice fed the control diet. The average weight of the right ovaries for the 3 groups was 4.03 milligrams, 3.86 milligrams, and 4.47 milligrams, respectively, while the average weight of the left ovaries was 3.72 milligrams, 4.03 milligrams, and 3.71 milligrams, respectively.

There was much variation in the weights of the ovaries from mice on the same diet. The right ovaries varied in weight from 0.7 to 7.1 milligrams in the Tween 20 dietary group; 1.2 to 6.7 milligrams in the Tween 60 group; and from 0.5 to 10.4 milligrams in the control group. The weight of

Table 39. Ovary weights of first generation females[a]

Tween 20		Tween 60		Control	
Right ovary	Left ovary	Right ovary	Left ovary	Right ovary	Left ovary
1.9	1.4	2.8	2.7	3.5	4.2
0.7	1.9	4.2	3.9	5.5	2.9
3.0	4.1	3.8	4.5	10.4	5.9
0.8	3.0	3.1	7.0	3.7	1.9
5.1	3.0	4.3	3.9	4.4	3.7
4.4	4.1	3.2	5.0	5.2	5.9
6.6	4.4	4.8	5.0	3.3	1.4
6.1	5.5	5.5	2.9	5.2	5.4
1.8	2.6	1.6	1.6	5.3	3.3
4.9	3.6	2.4	2.4	4.3	4.6
4.3	4.8	6.7	3.4	4.6	5.1
6.8	6.5	4.4	5.0	5.0	4.6
7.1	3.4	6.0	5.5	4.0	2.4
5.1	4.1		5.9	2.6	1.7
2.5	3.4	1.2	1.8	2.2	5.8
6.3	5.7			2.5	2.2
2.4	1.8			5.9	4.9
3.4	4.6			4.3	3.4
3.3	2.8			4.3	4.0
				5.1	4.2
				4.6	3.8
				6.6	3.3
				5.3	3.9
				1.6	2.9
				4.1	4.7
				4.7	4.2
				6.9	2.2
				4.1	4.7
				0.5	0.5
Total					
76.5	70.7	54.0	60.5	129.7	107.7
Average					
4.03	3.72	3.86	4.03	4.47	3.71

[a]Weights given in milligrams.

the left ovaries ranged from 1.4 to 6.5 milligrams in the Tween 20 group; from 1.6 to 7.0 milligrams in the Tween 60 group; and from 0.5 to 5.9 milligrams in the control mice. The difference in weights between the smallest and largest ovary was least in the Tween 60 group, being 5.8 milligrams, whereas the greatest difference between the average ovarian weight of mice on the different diets was only 0.22 milligrams. One could not attach any significance to the difference of the average ovarian weight of mice fed the different diets.

The average weight of the right ovaries was slightly greater than that of the left ovaries in the Tween 20 group (0.3 milligrams) and the control group (0.76 milligrams), but slightly less in the Tween 60 dietary group (0.17 milligrams).

As has been noted there was much variation in the weight of the ovaries of one mouse as compared with that of another. It was also interesting to note that a rather marked difference existed between the weight of the right and left ovary of many of the mice. It was not uncommon to find that one ovary weighed almost twice as much as the other and in one mouse on the Tween 20 diet the left ovary (3.0 milligrams) weighed almost four times as much as the right ovary (0.8 milligrams). Considering the variation in ovary weights, a much larger sample would be needed before significance could be attached to this difference in the average weights of the right and left ovaries.

The average weights of the ovaries of the mice used in this study are comparable to those recorded by Green (1957) for C57BL/6J strain of mice, and are in line with those reported by Soliman and Reineke (1952) for albino mice.

Testis Weights

Testis weights were taken from a sample of first generation males approximately 11 months of age. These weights are recorded in Table 40. The mice included 23 on the Tween 20 diet; 17 on the Tween 60 diet; and 20 on the control diet. The average weight of the right testes was 98.52 milligrams, 92.91 milligrams, and 93.31 milligrams respectively, while the average weight of the left testes was 96.76 milligrams, 93.26 milligrams, and 93.09 milligrams, respectively.

There was much variation in the weights of the testes from mice on the same diet. The right testes varied in weight from 72.4 to 131.8 milligrams in the Tween 20 dietary group; from 76.4 to 113.7 milligrams in the Tween 60 dietary group; and from 72.0 to 110.6 milligrams in the control group. The weight of the left testes ranged from 77.9 to 110.4 milligrams in the Tween 20 group; from 75.5 to 109.0

Table 40. Testis weights of first generation males[a]

Tween 20		Tween 60		Control	
Right testis	Left testis	Right testis	Left testis	Right testis	Left testis
86.5	93.6	106.3	104.7	96.5	99.5
109.8	106.7	104.1	95.6	86.1	88.0
106.4	107.6	100.7	101.7	101.1	93.6
106.0	108.3	85.0	87.6	80.0	88.5
108.6	110.4	79.5	84.5	89.0	84.5
96.7	103.6	82.3	90.0	102.4	95.0
107.5	101.2	93.2	94.1	74.9	71.3
102.3	94.6	100.1	96.1	88.9	90.3
112.1	108.0	79.1	88.4	107.4	106.6
95.8	93.2	103.4	109.0	90.7	95.9
72.4	77.9	97.5	96.2	91.9	83.0
107.2	101.4	86.4	86.6	95.9	91.6
85.4	80.2	86.1	97.3	87.1	91.3
104.2	107.3	89.4	76.8	102.2	105.1
111.6	103.1	113.7	108.2	98.3	94.7
91.0	86.4	96.2	93.1	80.1	78.5
103.0	97.7	76.4	75.5	103.6	101.5
95.0	98.2			107.6	107.6
77.6	79.7			72.0	92.6
74.6	80.6			110.6	102.7
84.6	85.7				
131.8	95.8				
96.1	104.3				
Total					
2266.00	2225.50	1579.40	1585.40	1866.30	1861.80
Average					
98.52	96.76	92.91	93.26	93.31	93.09

[a]Weights given in milligrams.

milligrams in the Tween 60 group; and from 71.3 to 107.6 milligrams in the control group of mice.

The variation was least in the left testes of the Tween 20 group, being 32.5 milligrams, whereas the greater difference between the average testicle weight of mice fed different diets was only 5.61 milligrams. The data were subjected to an analysis of variance and the F value obtained (1.06) indicated no significance. The difference of 5.61 milligrams was partly due to one very large testis (131.8 milligrams) found in the Tween 20 group which weighed 18.1 milligrams more than the next heaviest testis.

The average weight of the left testes was very close (within .35 milligrams) to the average weight of the right testes in the Tween 60 and control groups. Most of the difference between the average weights of the left and right testis in the Tween 20 group can be attached to the one exceptionally large testis, previously noted.

As in the case of the ovaries, both gonads in the same male are rarely of the same weight. The greatest difference between right and left testis in the same mouse is less than 40%, while the greatest difference in ovarian weights in the same female is nearly 400%.

As has been acknowledged previously, one cannot make valid comparisons between mice of different strains, but it is noteworthy that the weights of the testes recorded in this study are comparable to those reported by Maqsood and Reineke (1950) for mice of another strain.

Rate of Sperm Movement

The rate of sperm movement was calculated from semen obtained from a sample of first generation males. The procedure used in this study was described in the section under METHODS on Procedure for Checking Rate of Sperm Movement. The results are recorded in Tables 41, 42, and 43. The microscope used was calibrated and each large micrometer unit was equal to 0.14 mm.

Sperm were checked from 12 mice on the Tween 20 diet, from 12 mice on the Tween 60 diet, and from 13 mice on the control diet. The average time taken by sperm to move 0.14 mm. was 1.812 seconds, 1.612 seconds and 1.702 seconds for sperm from mice on Tween 20, Tween 60, and the control diet, respectively. There was much variation in the rate of sperm movement. It was common to find some sperm traveling twice as fast as other sperm from the same mouse. The average time required for sperm of mice within a dietary group to move 0.14 mm. varied about 0.8 seconds, whereas the greatest difference in the average time, between groups, was only 0.2 seconds.

It is quite probable that an even smaller difference

Table 41. Time, in seconds, required for sperm from mice fed <u>Tween</u> <u>20</u> to travel 0.14 millimeters[a]

Mouse											
2L'-5	1H'-1	1F^2-3	1F^2-4	1F^2-3	1R'-2	1H'-2	1H'-3	2M'-1	1H'-2	1K'-3	2F'-1
2.2	1.2	1.5	1.1	1.5	2.0	2.3	3.0	2.0	2.9	1.2	2.1
1.7	1.7	1.4	1.7	1.4	1.8	1.9	1.2	2.5	1.3	1.2	2.1
3.5	1.6	1.6	1.3	1.8	1.4	1.7	1.8	4.2	1.7	1.5	1.7
1.2	1.6	1.9	1.2	2.0	1.8	2.2	2.2	3.8	1.8	1.0	2.1
1.8	1.6	2.0	1.6	1.9	1.5	2.0	2.2	2.3	1.5	1.1	1.6
1.7	1.6	1.8	2.0	2.3	1.8	2.0	2.3	2.3	1.5	1.6	1.5
1.9	1.4	2.5	2.0	2.5	2.3	3.0	1.6	2.2	1.6	1.2	1.5
1.8	2.1	2.0	1.4	1.6	1.4	1.3	2.2	1.8	1.8	1.1	1.3
1.4	1.7	1.8	1.6	1.6	1.6	1.9	1.5	2.7	1.8	1.7	1.3
1.6	1.7	1.8	1.5	1.8	1.6	1.9	1.7	1.5	2.3	1.6	1.4
Total											
18.8	16.2	18.3	15.4	18.4	17.2	20.2	19.7	25.3	18.2	13.2	16.6
Average											
1.88	1.62	1.83	1.54	1.84	1.72	2.02	1.97	2.53	1.82	1.32	1.66

[a]Average time for sperm of mice on <u>Tween</u> <u>20</u> - 1.812 seconds.

Table 42. Time, in seconds, required for sperm from mice fed <u>Tween 60</u> to travel 0.14 millimeters[a]

Mouse											
1H'-3	1E'-2	1K'-4	2H'-1	2F'-3	1G'-4	G'-2	1R'-3	1V'-3	1N'-1	10'-3	1F'-2
1.9	1.3	1.1	1.1	1.9	2.2	1.1	1.8	1.6	1.3	1.8	1.2
2.2	1.2	1.4	1.8	1.5	2.2	1.9	1.4	1.5	1.7	1.1	1.3
1.9	2.6	1.5	1.4	2.0	2.1	1.9	1.3	1.8	1.2	1.9	1.2
1.9	2.0	1.3	1.5	1.2	1.7	2.4	1.3	1.2	1.2	1.8	1.8
2.5	1.2	1.6	1.3	1.4	1.2	1.5	1.4	1.4	1.8	1.8	1.8
1.7	1.5	1.8	1.3	1.3	1.5	2.1	1.3	1.4	1.7	1.5	1.6
2.0	1.6	1.4	2.3	1.7	1.6	1.6	1.2	1.8	1.4	1.2	1.7
1.7	1.7	1.3	1.9	1.7	1.7	1.7	1.4	1.7	1.2	1.6	1.1
2.0	1.8	1.8	1.3	1.4	1.5	3.4	1.2	1.8	1.3	1.6	1.4
2.3	1.6	1.7	1.6	1.5	2.2	1.2	1.1	1.4	1.3	1.6	2.1
Total											
20.1	16.5	14.9	15.5	15.6	17.9	18.8	13.4	15.6	14.1	15.9	15.2
Average											
2.01	1.65	1.49	1.55	1.56	1.79	1.88	1.34	1.56	1.41	1.59	1.52

[a]Average time for sperm of mice on <u>Tween 60</u> - 1.612 seconds.

Table 43. Time, in seconds, required for sperm from mice fed the control diet to move 0.14 millimeters[a]

Mouse												
2E'-1	1O'-1	2L'-1	2P'-2	2N'-2	1O'-4	$1F^2$-1	$1F^2$-2	1O'-2	2H'-2	2O'-1	2M'-2	2L'-2
1.0	1.1	2.2	1.6	1.6	3.4	1.2	1.6	1.5	2.4	1.1	1.3	1.5
1.5	2.3	1.1	1.4	1.4	1.7	.9	1.0	1.2	.9	1.2	1.2	1.3
2.5	2.0	1.8	1.7	1.8	1.7	.7	1.7	1.1	1.2	1.9	1.3	1.6
1.1	3.0	1.5	2.1	2.3	1.5	1.5	1.4	1.3	.9	1.7	1.1	1.2
2.3	1.6	1.8	2.2	1.6	1.5	1.0	2.5	1.0	1.4	1.9	1.8	1.5
2.5	1.9	2.4	1.0	1.8	3.0	1.7	2.0	1.5	1.4	1.3	1.6	1.3
2.2	1.7	2.7	1.1	1.3	1.7	1.2	1.9	1.4	1.6	1.7	1.9	1.3
1.8	2.0	2.0	1.6	2.7	2.4	1.5	1.6	1.4	1.5	1.6	1.5	1.1
1.6	1.2	2.2	1.8	2.3	1.8	1.2	1.6	1.2	1.5	1.7	1.4	1.2
2.0	1.6	2.6	1.6	1.4	1.2	1.4	1.5	1.3	1.1	1.3	2.1	1.4
Total												
18.5	18.4	20.3	16.1	18.2	19.9	12.3	16.8	12.9	13.9	15.4	15.2	13.4
Average												
1.85	1.84	2.03	1.61	1.82	1.99	1.23	1.68	1.29	1.39	1.54	1.52	1.34

[a]Average time for sperm of mice on control diet - 1.702.

between groups would exist if a larger sample of sperm had been checked. The average time determined for sperm of mouse 2M'-1 of the Tween 20 group was considerably greater than for sperm of other mice in the same group; almost twice as fast, for example, as sperm from 1K'-3 of the same group.

The rate of sperm movement of the C57BL/6 strain mice, as determined in this study, was about 4.9 mm. per minute. No significant effect upon the rate of sperm movement was noted by feeding Tween 20 or Tween 60 at a 10% concentration. The writer has not yet found comparable data in the literature, so can make no comparison with sperm rate from other mice.

Sex of Offspring at Weaning

In most cases, record was made of the sex of the mice that were weaned. The data in Table 44 are based on the sex of the mice at the time of weaning.

In each group, with the exception of the Tween 60 and control third generation mice, the number of males was equal to or greater than the number of females weaned. On the basis of the information of this study, it appears that the C57BL/6 mice tend to wean more males than females. Taking all mice together there are approximately 112 males weaned for each 100 females.

Table 44. Sex of offspring at weaning

	Tween 60		Tween 20		Control	
Generation	Males	Females	Males	Females	Males	Females
First	263	243	268	268	471	402
Second	77	69	151	107	105	82
Third	62	54	74	83	64	68
Total	402	366	493	458	640	552

Grand total: males - 1535

females - 1376

Time at Which Litters Were Delivered

Since the cages of pregnant females were checked each morning and evening, it was possible to tell whether the offspring were born between the hours of 8 A.M. and 9 P.M. or between 9 P.M. and 8 A.M. The data as recorded for treatment groups and generations are found in Table 45.

The data revealed that variations existed from one group to another, and that there was no definite pattern. Taking all groups together, 251 litters were delivered between the hours of 8 A.M. and 9 P.M. and 281 delivered between the hours of 9 P.M. and 8 A.M. The information found here would suggest that mice are about as likely to deliver during one period as another. This is in contrast to information found in Snell (1941) which indicated that births most commonly occur at night with the maximum number being between midnight and 4 A.M.

Table 45. Time at which litters were delivered

Generation	Tween 20		Tween 60		Control	
	8AM-9PM	9PM-8AM	8AM-9PM	9PM-8AM	8AM-9PM	9PM-8AM
First	39	46	46	34	75	56
Second	23	35	22	20	21	24
Third	8	26	9	13	8	17
Total	70	107	77	77	104	97

Grand total: 8AM-9PM - 251

9PM-8AM - 281

SUMMARY AND CONCLUSIONS

Investigations in this study were undertaken to determine what influence long term continuous feeding of the emulsifiers Tween 20 (polyoxyethylene sorbitan monolaurate) and Tween 60 (polyoxethylene sorbitan monostearate) might have upon the reproductive performance of C57BL/6 strain mice when fed at the 10% level. The basic diet was Purina Mouse Breeder Chow.

Three generations of mice were involved in most of the study. There were 7 breeding groups in the first generation which included (a) a control group of mice, (b) groups in which both parents were on a Tween diet, and (c) groups in which one parent was on the control diet and the other on a Tween diet. Only those groups in which both parents were on the same diet, either control or experimental, were continued for the second and third generations. Four litters of mice were delivered from brother-sister matings by the first and second generation mice and three litters by the third generation mice.

Data relative to number born, number dead at birth, number alive at 4 days and number weaned were analyzed by the analysis of variance procedure. Data relative to weaning weights were analyzed by the analysis of covariance procedure.

One of the findings during the study was that weaning weights of offspring whose mothers were fed one of the Tween diets were significantly less ($P < .01$) than those of mothers fed the control diet. After litters were adjusted to a common size, their weights were 47.53 grams, 46.40 grams and 57.70 grams, respectively, for mice on the Tween 20, Tween 60, and control diets. It was also determined that the decreased weaning weight was due to a poorer weight gain in the experimental offspring during the third week of life. Of the litters for which data were taken, the average weight gain between the ages of 4 and 14 days was approximately 4 grams per mouse regardless of diet. However, between the ages of 14 and 21 days mice on the control diet averaged a gain of 2.8 grams compared with an average gain of less than 1.8 grams for mice fed the experimental diets.

Mice fed the Tween 60 diet weaned significantly smaller ($P < .05$) litters than did mice fed the control diet. Because of a relatively small number of entries, it was difficult to test accurately data relative to the number born dead. However, in this study, it was found that females fed the Tween 60 diet delivered significantly more ($P < .05$) offspring dead at birth than did mice on either the control or Tween 20 diets. There were 95 young born dead to those groups of mice in which both parents were fed the Tween 60 diet compared to 31 and 43 born dead to parents on the

Tween 20 and control diets, respectively.

The analyses revealed that the litter number had a significant effect upon the number of offspring born, the number of offspring alive at 4 days, and the number weaned. The second or third litter was typically the largest in each of the above cases. Data suggested that females fed the experimental diets tend to produce their largest litter at a later age than do females on the control diet. Feeding of the Tween compounds did not have any significant effect upon the weight of adult mice, upon the weight of gonads of adult mice, or upon the rate of sperm movement. The average weight of male mice approximately 1 year of age was slightly more than 32 grams and that of females was about 30.5 grams. Testes averaged between 92 and 98 milligrams each and the average ovary weight was between 3.7 and 4.5 milligrams. Sperm of the C57BL/6 mice moved at the average rate of 4.9 millimeters per minute.

It was also found during the study that approximately 112 males were weaned for each 100 females and that almost as many litters were delivered between the hours of 8 A.M. and 9 P.M. as between the hours of 9 P.M. and 8 A.M.

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